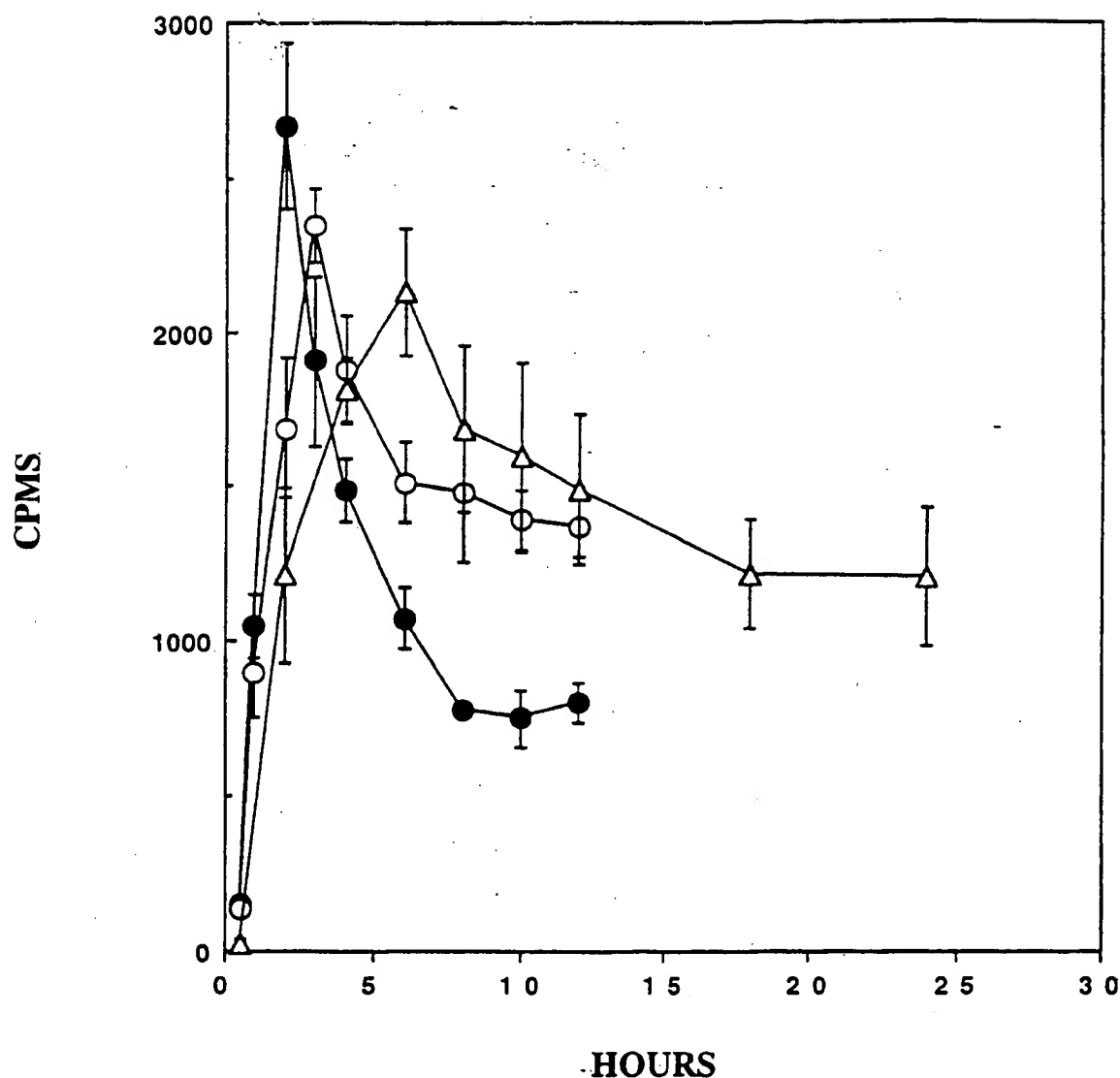


FIGURE 1

EFFECT OF TEMPERATURE ON THE SPECIFIC
BINDING OF 5 nM $^3\text{H}\text{-E}_2$ TO MTW9/PL2 CELLS

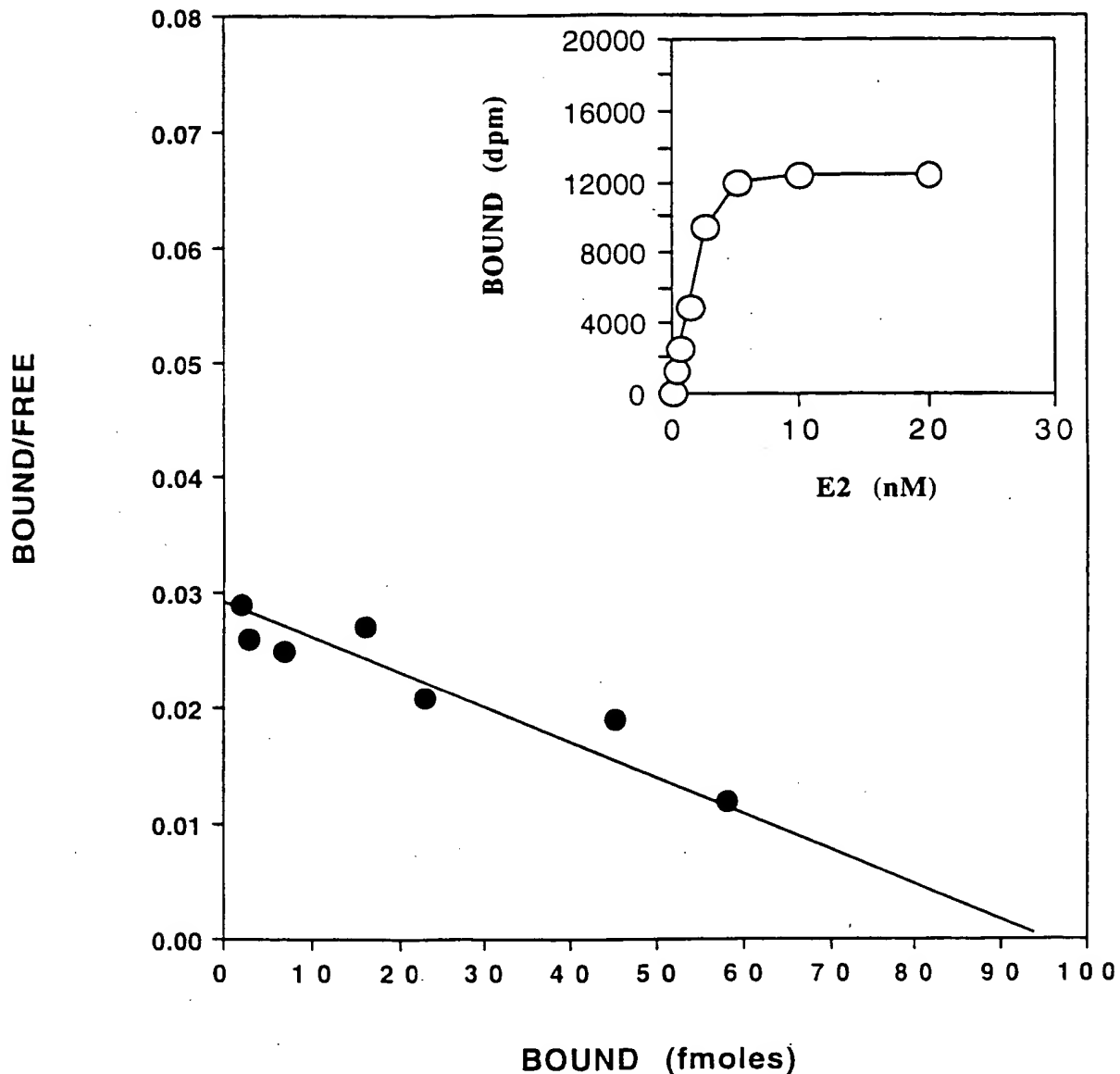


The kinetics are shown (\pm SD of triplicates) at 37°C (closed circles), 23°C (open circles), and at 4°C (open triangles).

Specific binding was determined in phenol red-free D-MEM/F-12. Each assay sample contained 300,000 CPM and 1.0×10^6 cells.

FIGURE 2

EFFECT OF CONCENTRATION ON THE SPECIFIC BINDING OF $^3\text{H}\text{-E}_2$ TO MTW9/PL2 CELLS AND A SCATCHARD ANALYSIS OF THE BINDING

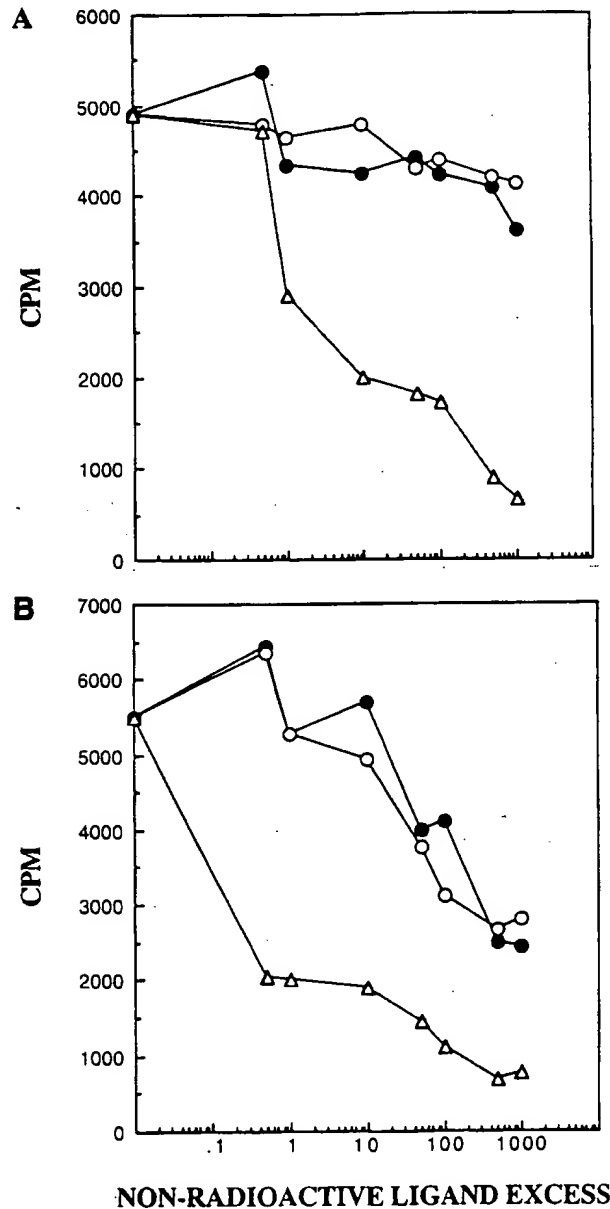


Scatchard analysis of $^3\text{H}\text{-E}_2$ binding (closed circles) was conducted using the traditional method with labeled-unlabeled mixtures of hormone and DES (100-fold excess) over the concentration range 37 pM to 5.0 nM $^3\text{H}\text{-E}_2$. In both experiments, 5 nM $^3\text{H}\text{-E}_2$ was 300,000 DPM. Each assay sample contained 1.0×10^6 cells.

INSERT: The insert shows a separate experiment in which the effect of $^3\text{H}\text{-E}_2$ concentration was measured on specific binding (DPM) after 2 h at 37°C in phenol red-free D-MEM/F-12.

FIGURE 3

EFFECT OF OTHER STEROID HORMONES ON THE BINDING OF $^3\text{H}\text{-E}_2$ TO MTW9/PL2 CELLS

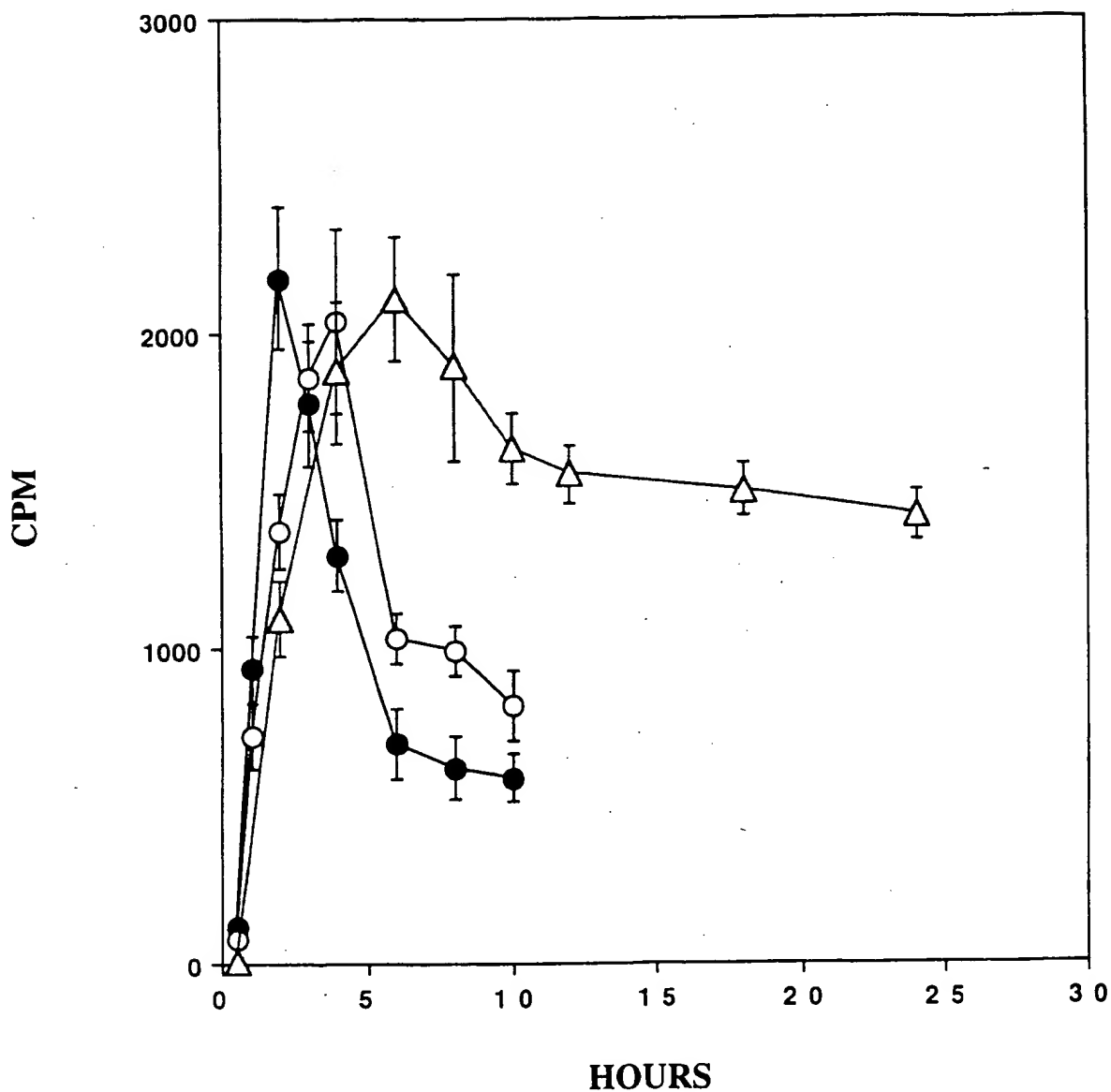


(A) shows the effects of unlabeled DES (open triangles), unlabeled DHT (open circles), and unlabeled T (closed circles).

(B) shows the effects of unlabeled DES (open triangles), unlabeled progesterone (open circles), and unlabeled cortisol (closed circles).

FIGURE 4

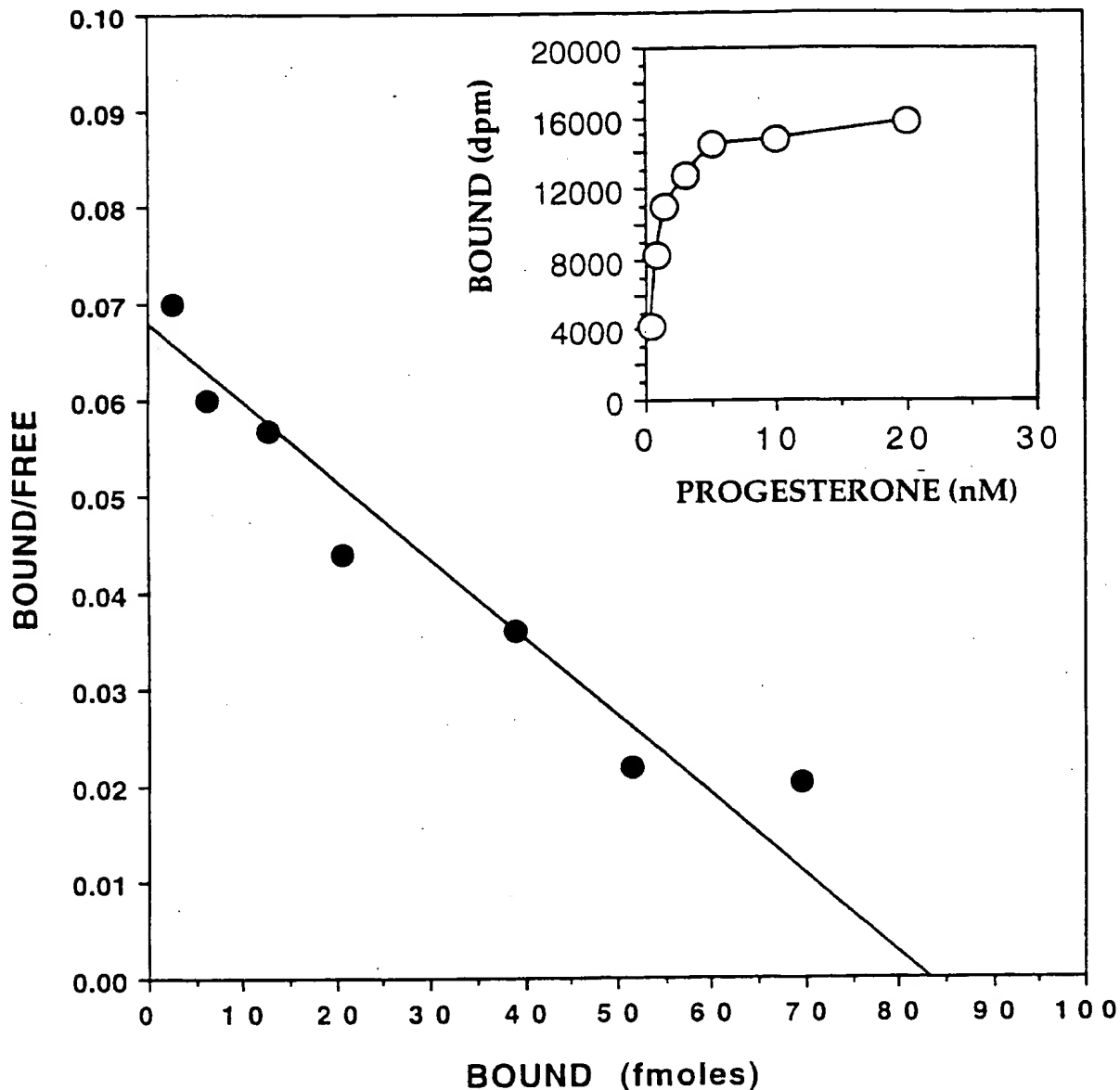
EFFECT OF TEMPERATURE ON THE SPECIFIC
BINDING OF 5 nM ^3H -PROGESTERONE TO
MTW9/PL2 CELLS



The kinetics are shown (SD of triplicates) at 37° C (closed circles), 23° C (open circles), and at 4° C (open triangles). Specific binding was determined in phenol red-free D-MEM/F-12. Each assay sample contained 287,000 CPM ^3H -progesterone and 1.0×10^6 cells.

FIGURE 5

**EFFECT OF CONCENTRATION ON THE SPECIFIC
 BINDING OF ^3H -PROGESTERONE TO MTW9/PL2 CELLS**

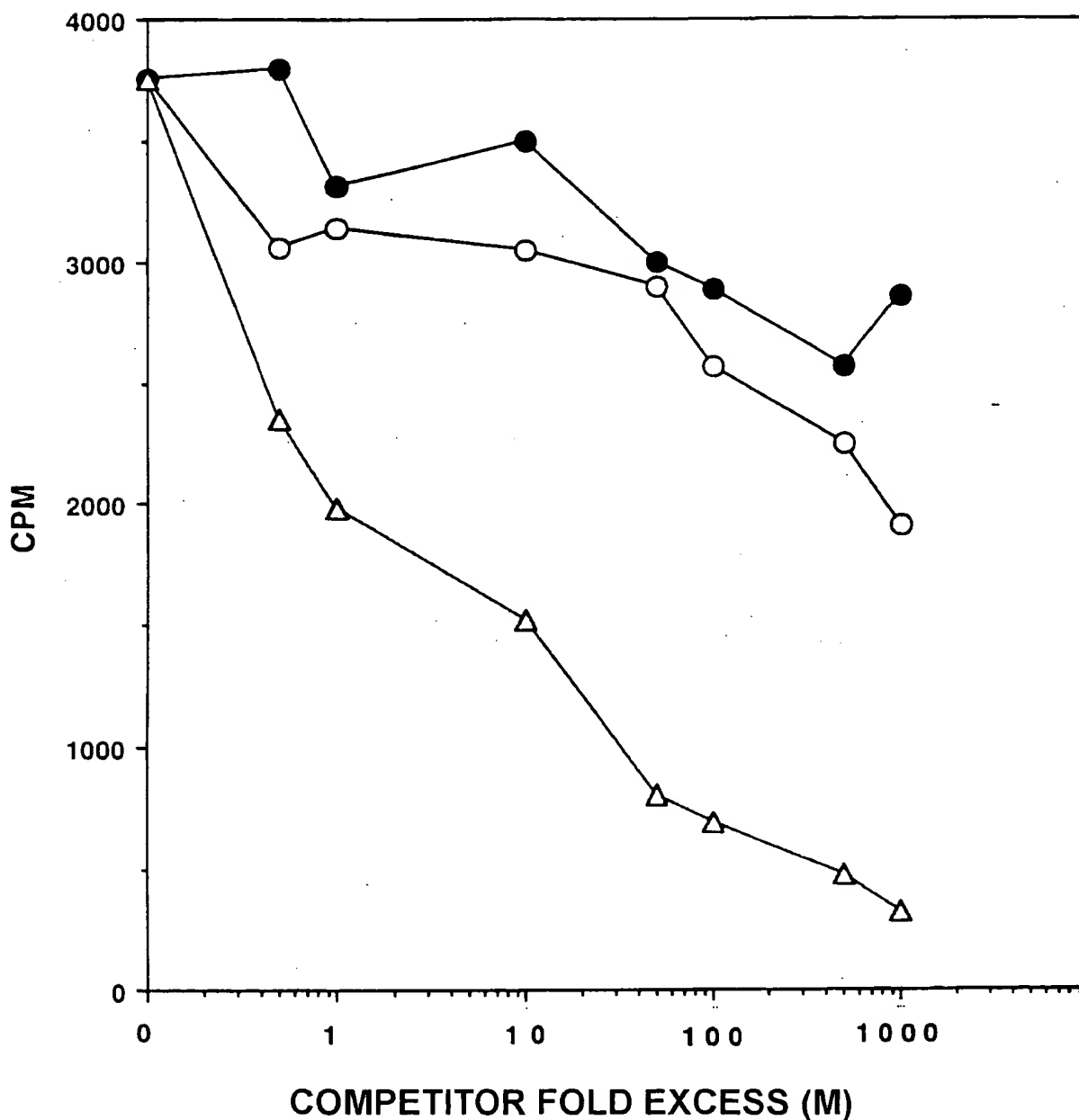


A Scatchard analysis of ^3H -progesterone binding (closed circles) was conducted using the traditional method with labeled-unlabeled mixtures of hormone and R5020 (100 fold excess) over the concentration range 37 pM to 5.0 nM ^3H -progesterone. In both experiments, 5.0 nM ^3H -progesterone was 287,000 CPM. Each assay sample contained 1.0×10^6 cells.

INSERT: The insert shows a separate experiment in which the effect of ^3H -progesterone concentration was measured on specific binding (bound dpm) after 2 h at 37°C in phenol red-free D-MEM/F-12.

FIGURE 6

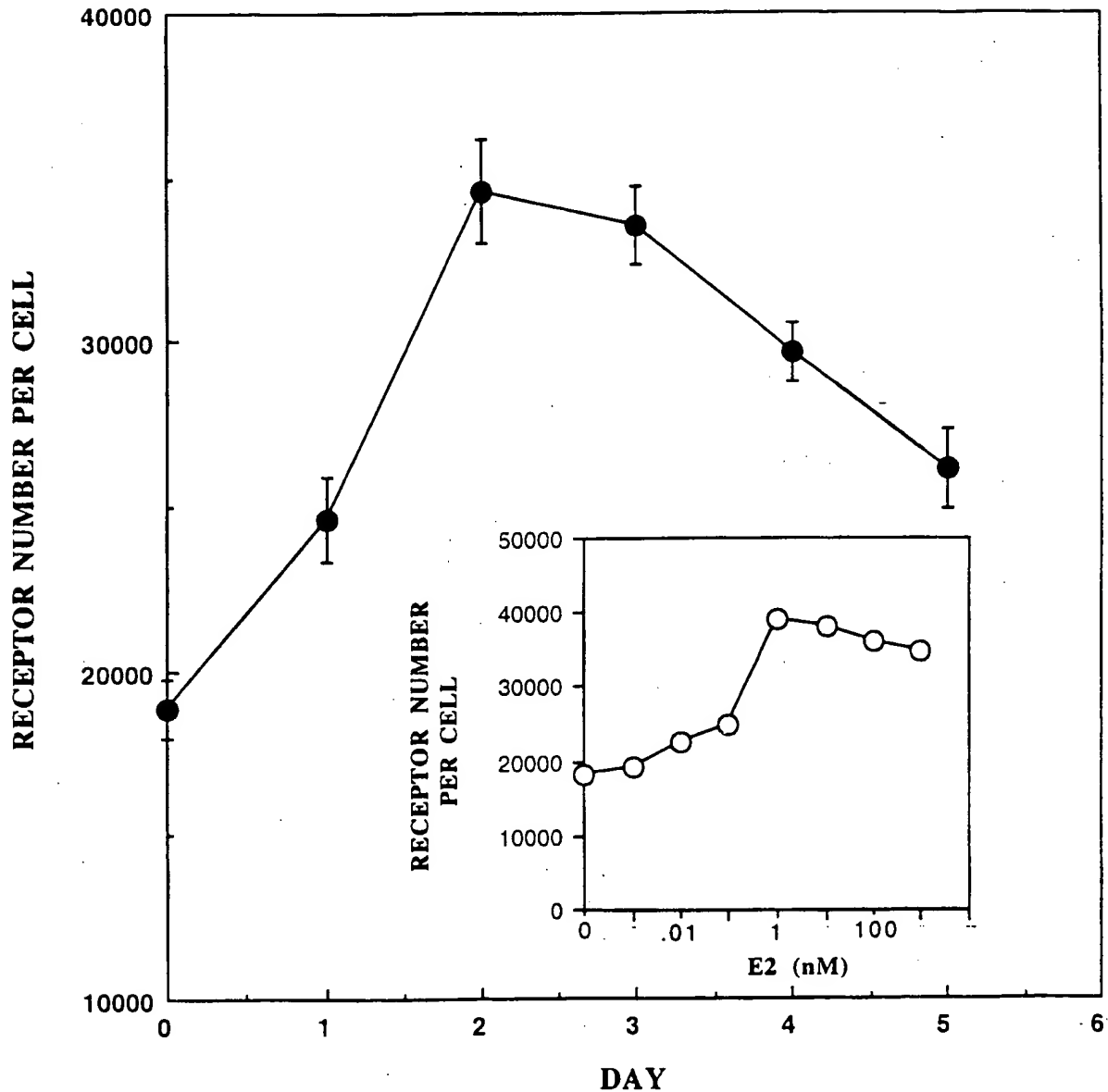
EFFECT OF STEROID HORMONES ON THE BINDING OF ^3H -PROGESTERONE TO MTW9/PL2 CELLS



The cells were incubated at 37°C for 2 h in the presence of 5 nM ^3H -progesterone (287,000 CPM) alone or in the presence of the labeled hormone plus the designated fold excess (M) of unlabeled R5020 (open triangles), unlabeled DHT (open circles), or unlabeled T (closed circles). Each assay sample contained 1.0×10^6 cells.

FIGURE 7

EFFECT OF E_2 ON THE PROGESTERONE RECEPTOR
CONTENT OF MTW9/PL2 CELLS



Each specific binding presented is the average of triplicate incubations \pm SD (closed circles).

INSERT: The insert shows the effect of E_2 concentration in the culture medium for 2 d prior to the assay of progesterone receptors (open circles).

FIGURE 8

**WESTERN IMMUNOBLOTTING
OF STEROID HORMONE RECEPTORS**

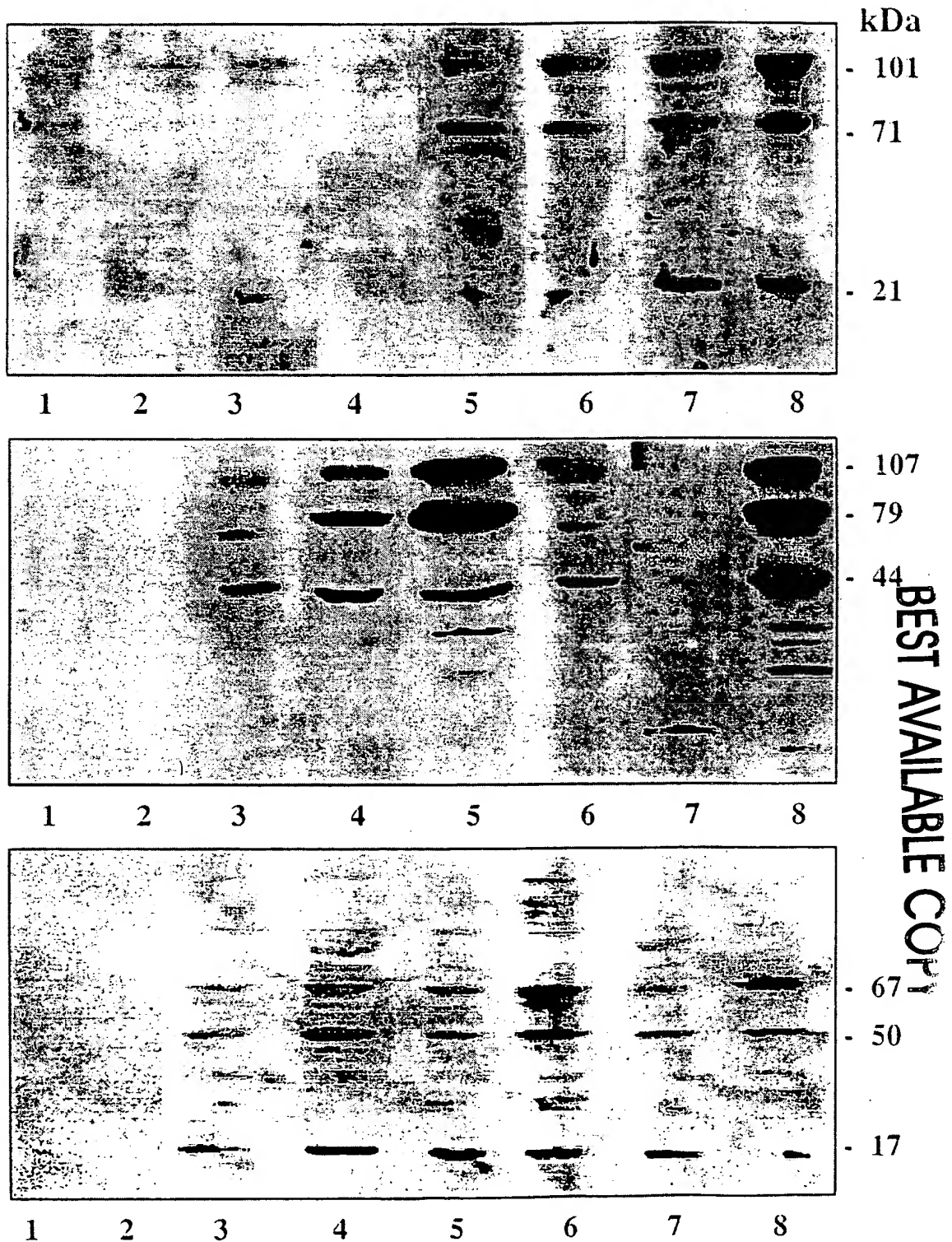
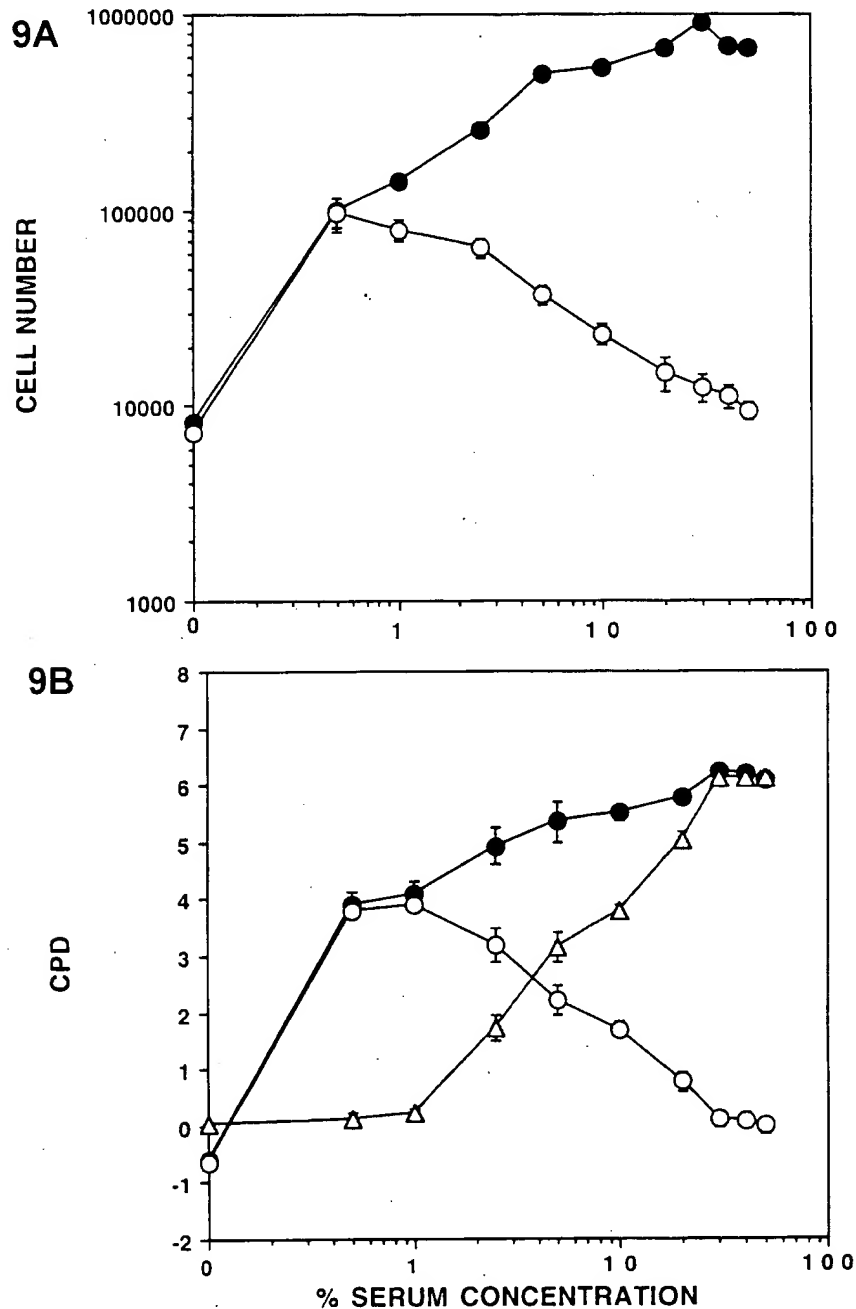


FIGURE 9

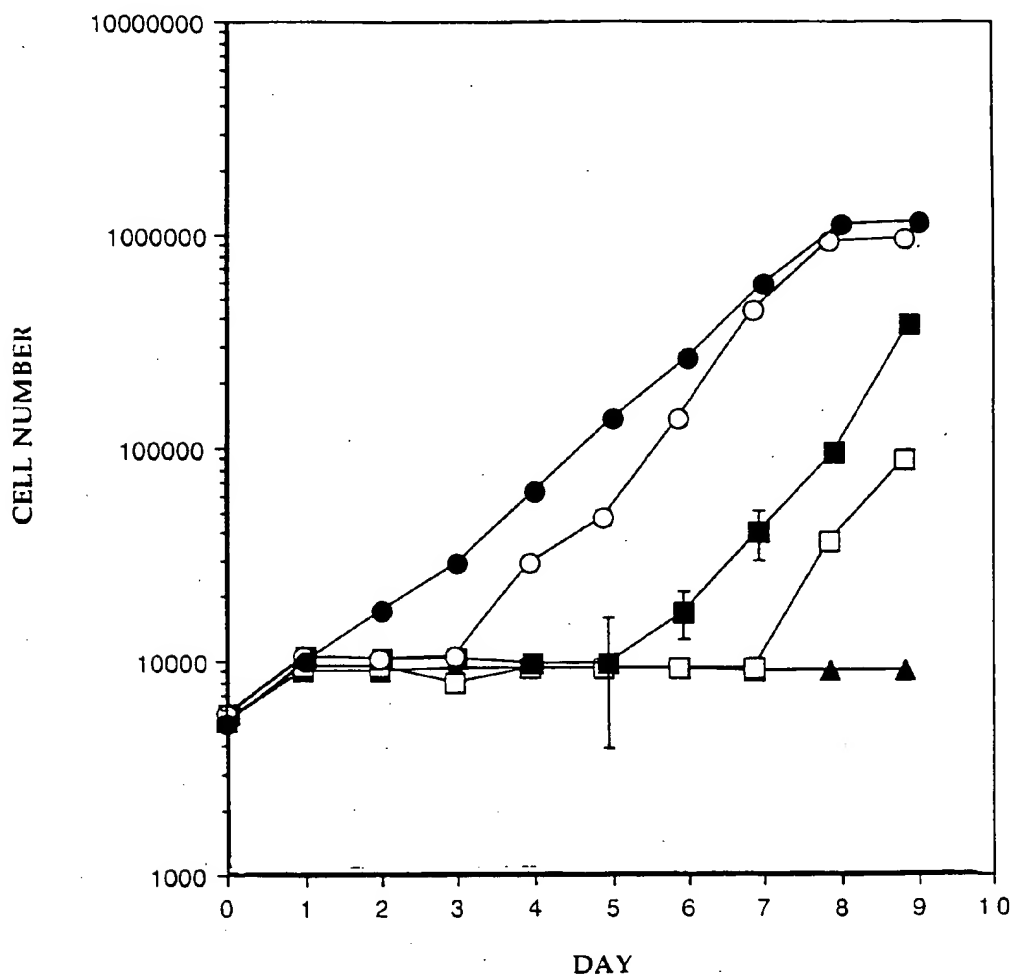
MTW9/PL2 CELL GROWTH IN 50% CDE - HORSE SERUM



- A: DATA EXPRESSED AS CELL NUMBER AFTER 7 DAYS**
 Growth with 1.0×10^{-8} M E (closed circles) and without hormone (open circles) in medium containing the designated concentrations of serum.
- B. DATA IN (A) EXPRESSED AS CPD**
 The symbols indicate the same conditions as (A) except the open triangles show CPD differences between growth in dishes with and without the hormone (Difference = estrogenic effect on growth).

FIGURE 10

**MTW9/PL2 CELL GROWTH IN 50% CDE - HORSE SERUM WITH
ESTROGENS ADDED AT VARIOUS TIMES AFTER SEEDING**

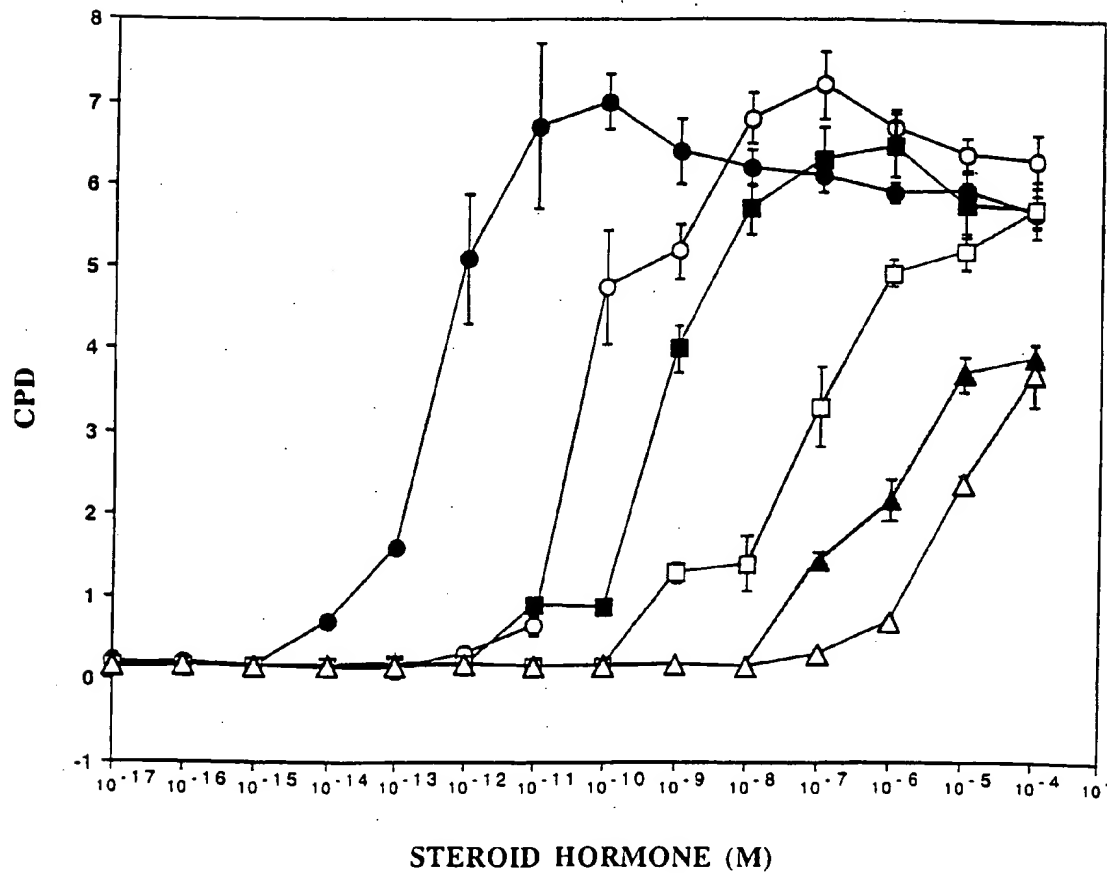


LEGEND:

Control growth in the absence of exogenous estrogen is shown by (triangles). In other dishes, 1.0×10^{-8} M E_2 was added at the beginning of the experiment (closed circles), after 48 h (open circles), after 96 h (closed squares), or after 144 h (open squares).

FIGURE 11

STEROID HORMONE DOSE RESPONSE EFFECTS WITH
MTW9/PL2 CELLS IN 50% CDE - HORSE SERUM

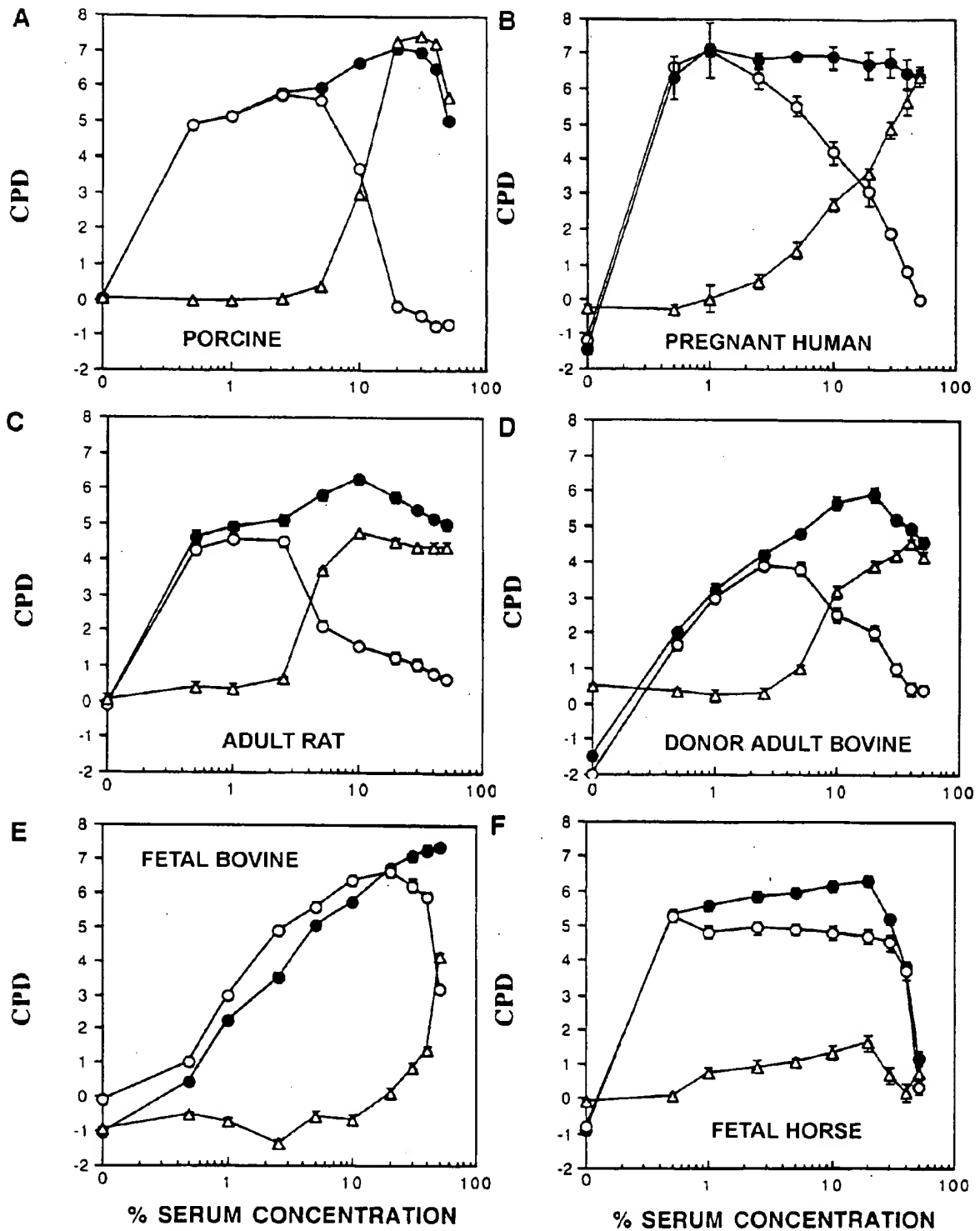


LEGEND:

Closed circles = E₂
Open circles = E₁
Closed squares = E₃
Open squares = Progesterone
Closed triangles = DHT
Open triangles = T

FIGURE 12

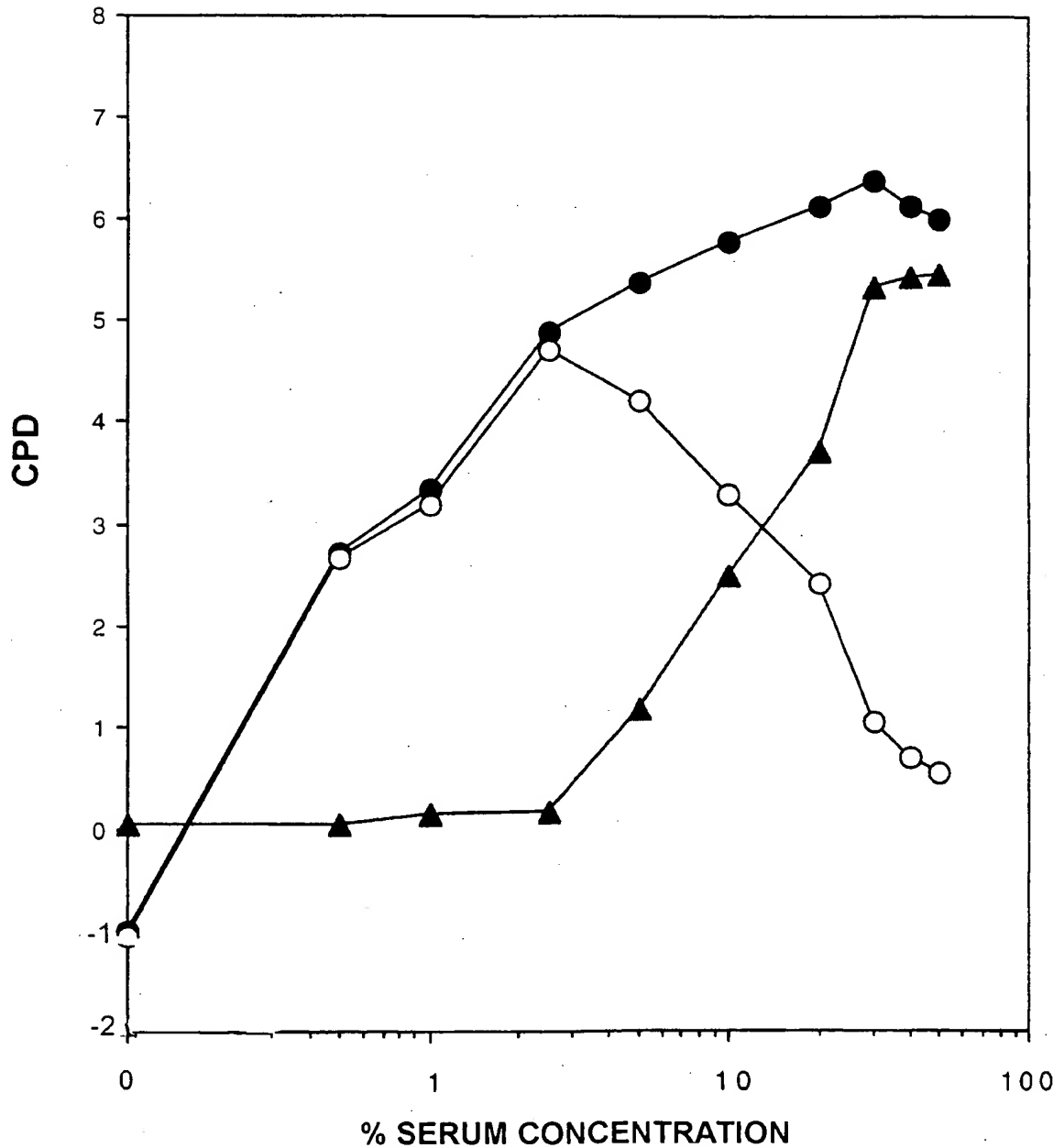
**MTW9PL2 CELL GROWTH IN CDE SERUM
 FROM DIFFERENT SPECIES**



LEGEND: Open circles = -E₂
 Closed circles = +E₂
 Open triangles = Estrogenic effect

FIGURE 13

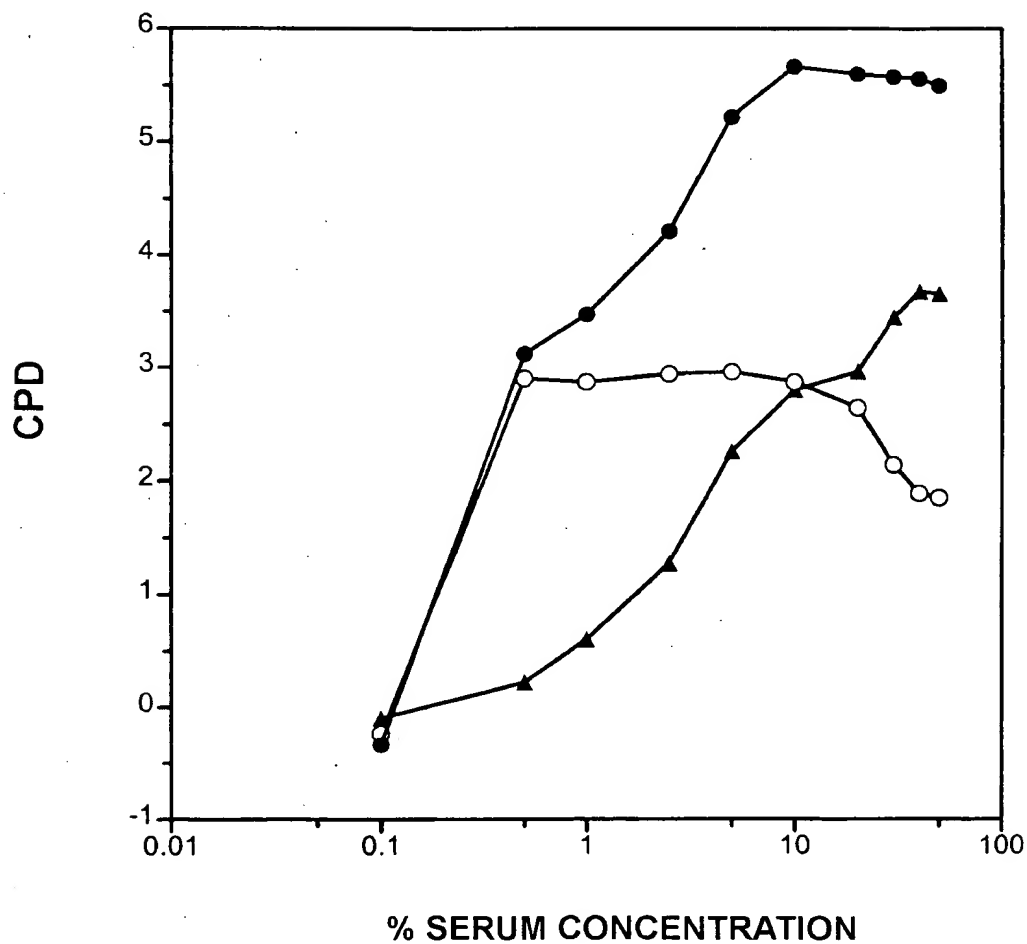
CDE HORSE SERUM TITRATION
GH4C1 CELLS



LEGEND: —●— = + E₂
—○— = - E₂
—▲— = Estrogenic effect

FIGURE 14

ZR-75-1 CELLS IN CDE - HORSE SERUM \pm 10 nM E_2



LEGEND:

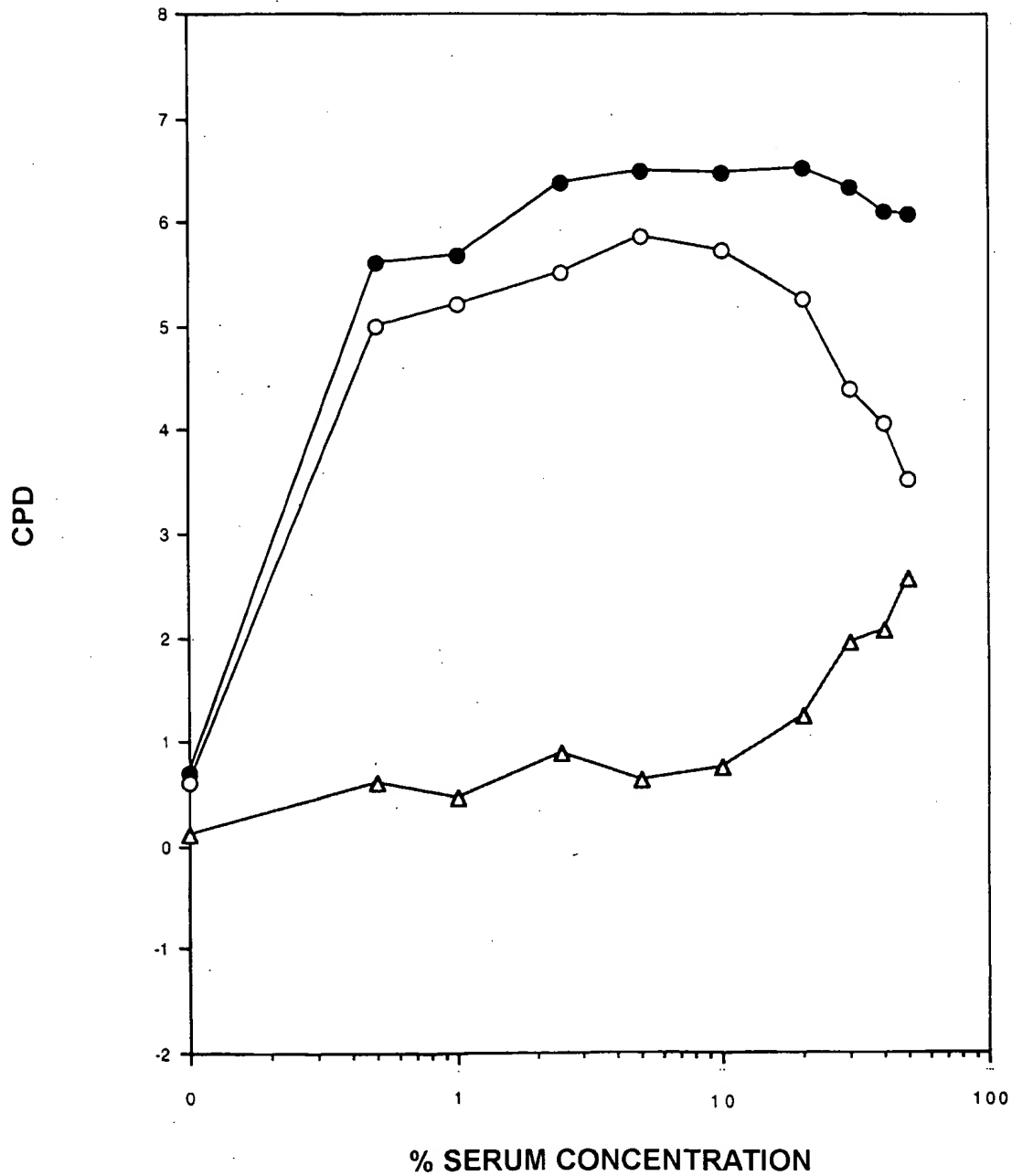
Closed circles = +E₂

Open circles = -E₂

Closed triangles = Estrogenic effect

FIGURE 15

MCF7A CELL GROWTH IN CDE - HORSE SERUM $\pm E_2$

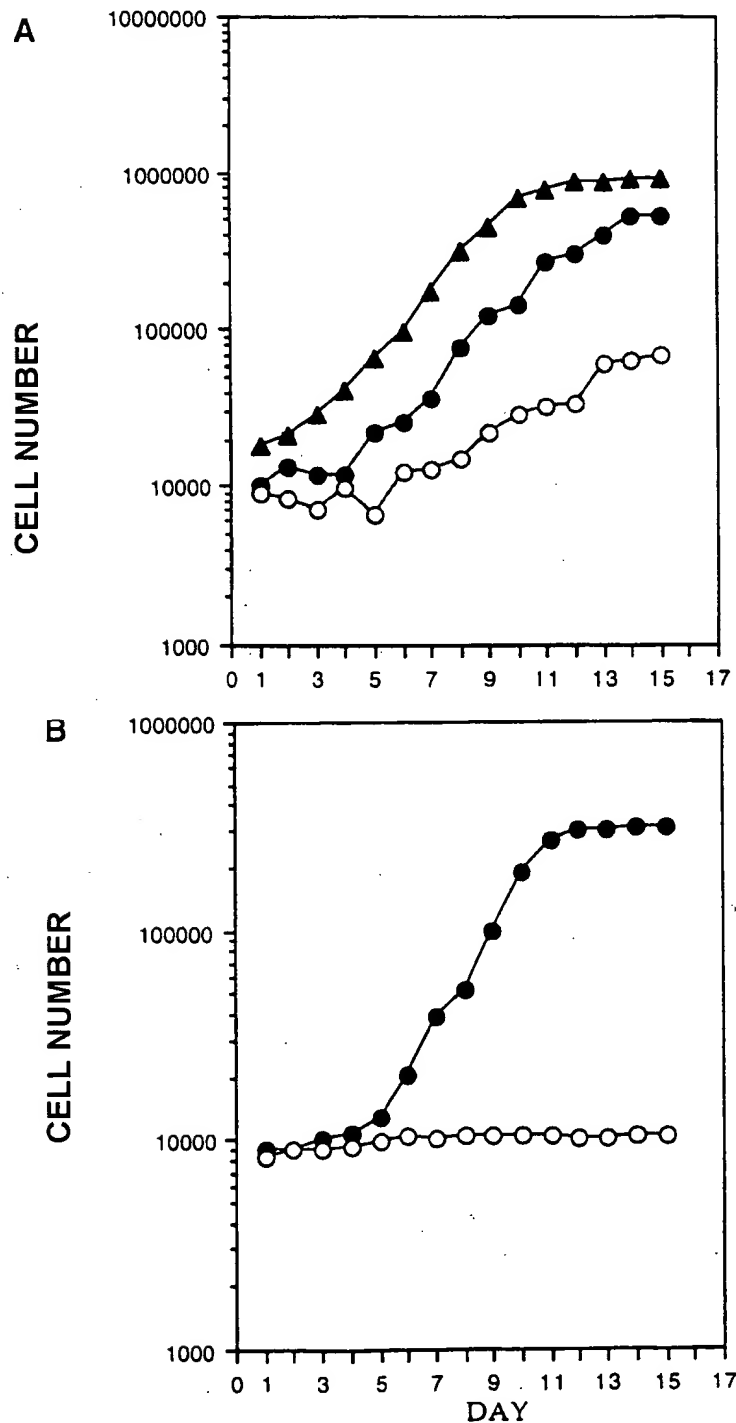


LEGEND:

Closed circles = +E₂
Open circles = -E₂
Closed triangles = Estrogenic effect

FIGURE 16

GROWTH KINETICS OF T47D HUMAN BREAST CANCER CELLS IN CDE - HORSE SERUM ± 10 nM E_2

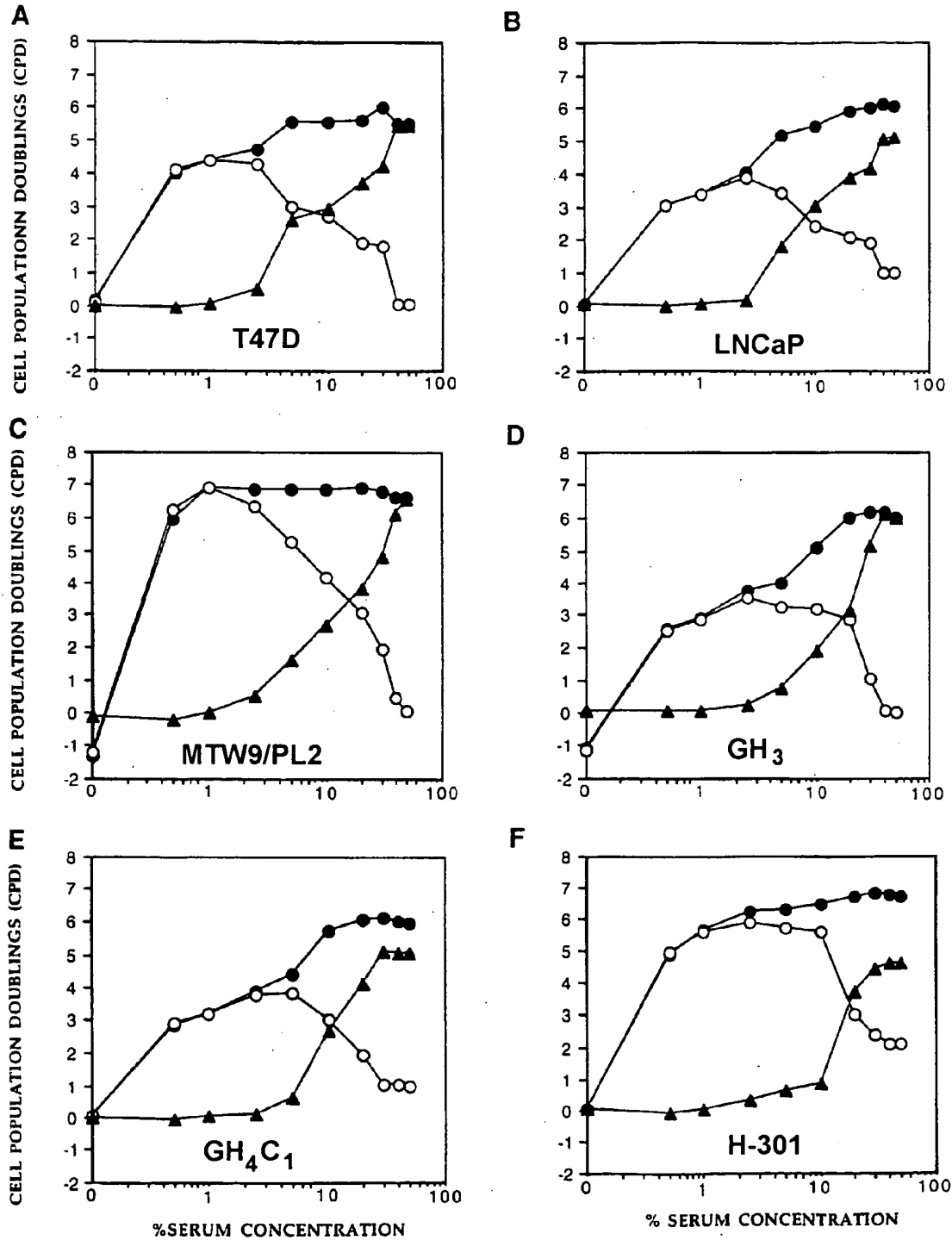


(A) The growth of the cells in medium with 20% (v/v) serum with 10 nM E_2 (closed circles) and without the steroid (open circles). As comparison, growth is shown in medium containing 10% (v/v) FBS (triangles).

(B) T47D cell growth kinetics in medium with 50% (v/v) serum with E_2 (closed circles) and without the steroid (open circles).

FIGURE 17

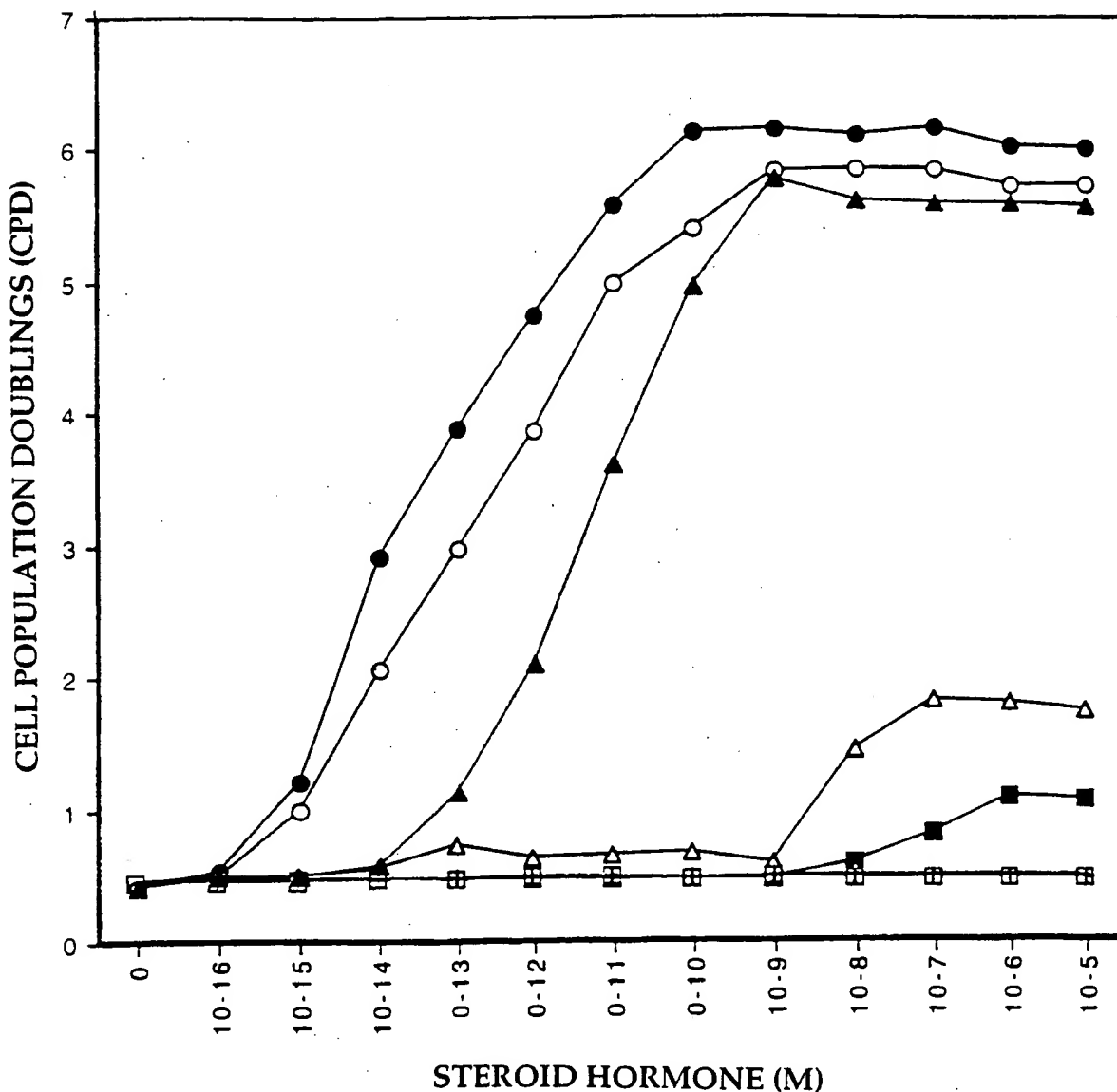
GROWTH OF HUMAN & RODENT CELL LINES IN 50% CDE - HORSE SERUM $\pm E_2$ (10 nM)



LEGEND: Closed circles = Medium with 10 nM E_2
 Open circles = Medium without E_2
 Triangles = Estrogenic effect

FIGURE 18

**DOSE RESPONSE OF STEROID HORMONES
 WITH T47D CELLS IN 50% CDE - HORSE SERUM**



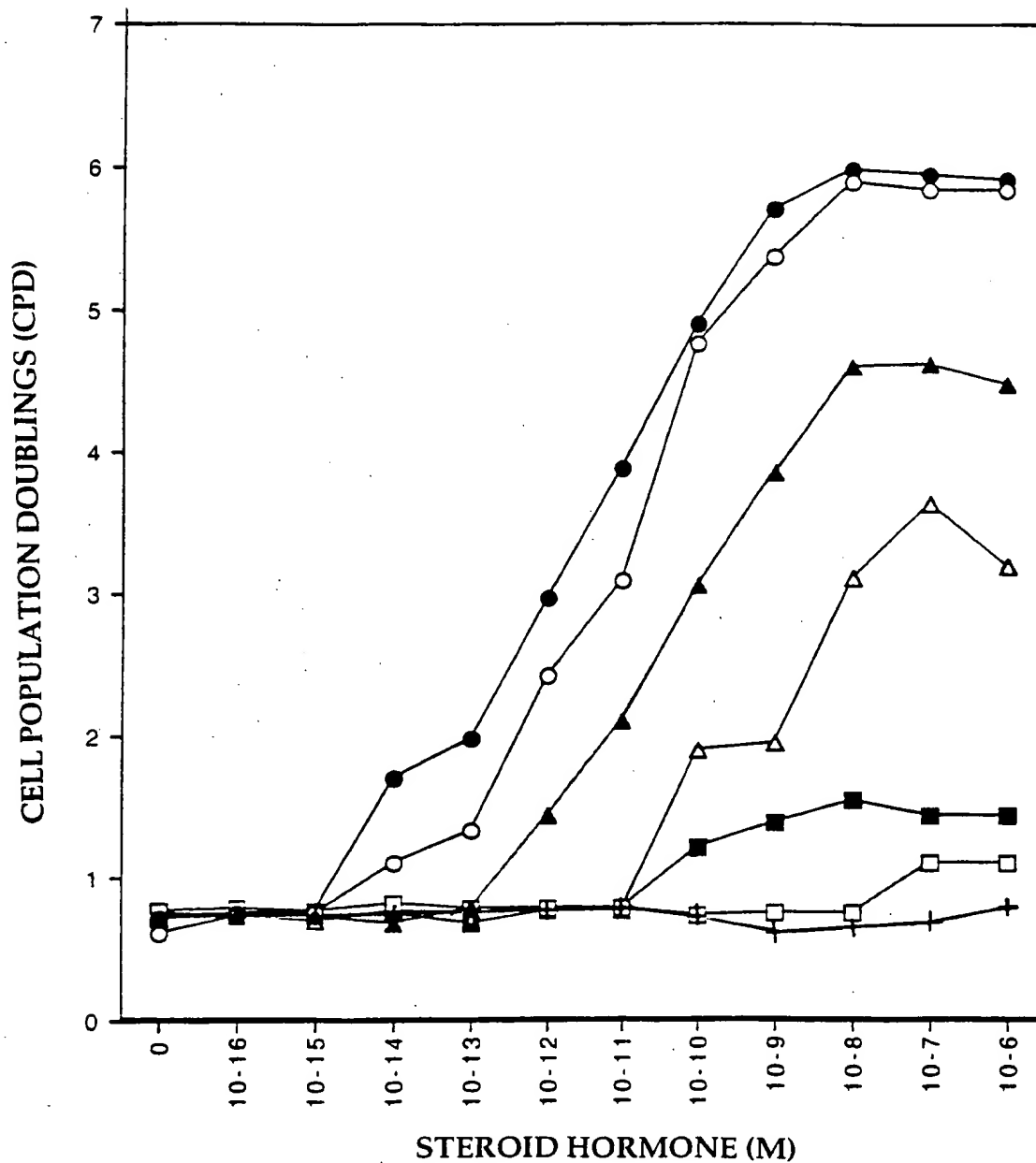
LEGEND:

Growth after 14 days is shown in response to:

- Closed circles = E₂
- Open circles = E₁
- Closed triangles = E₃
- Open triangles = DHT
- Closed squares = Testosterone
- Open squares = Progesterone
- Crosses = Cortisol

FIGURE 19

**DOSE RESPONSE OF STEROID HORMONES
 WITH GH₄C₁ CELLS IN 50% CDE - HORSE SERUM**



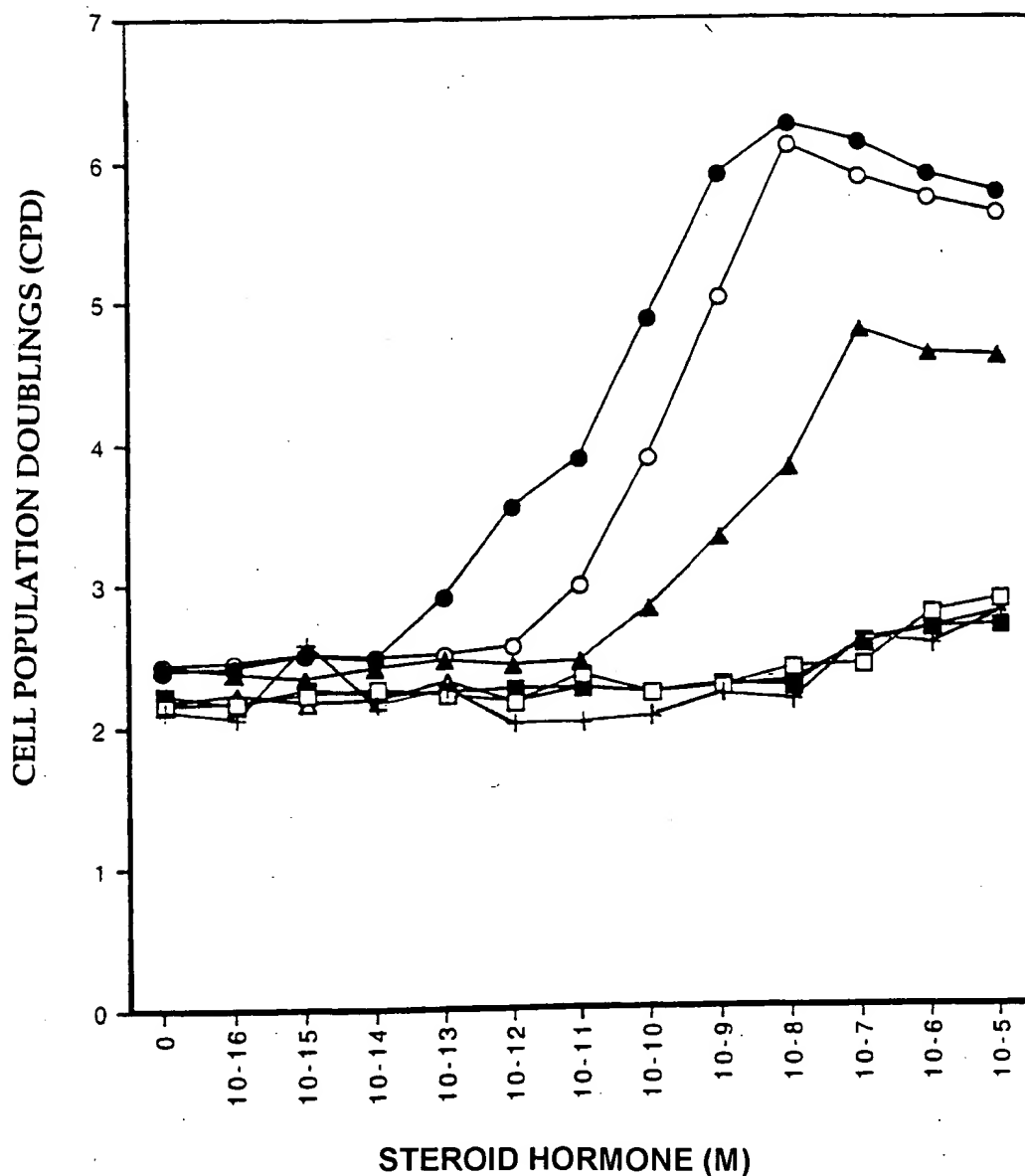
LEGEND:

Growth after 11 days is shown in response to:

- Closed circles = E₂
- Open circles = E₁
- Closed triangles = E₃
- Open triangles = DHT
- Closed squares = Testosterone
- Open squares = Progesterone
- Crosses = Cortisol

FIGURE 20

DOSE RESPONSE OF STEROID HORMONES
WITH H-301 CELLS IN 50% CDE - HORSE SERUM



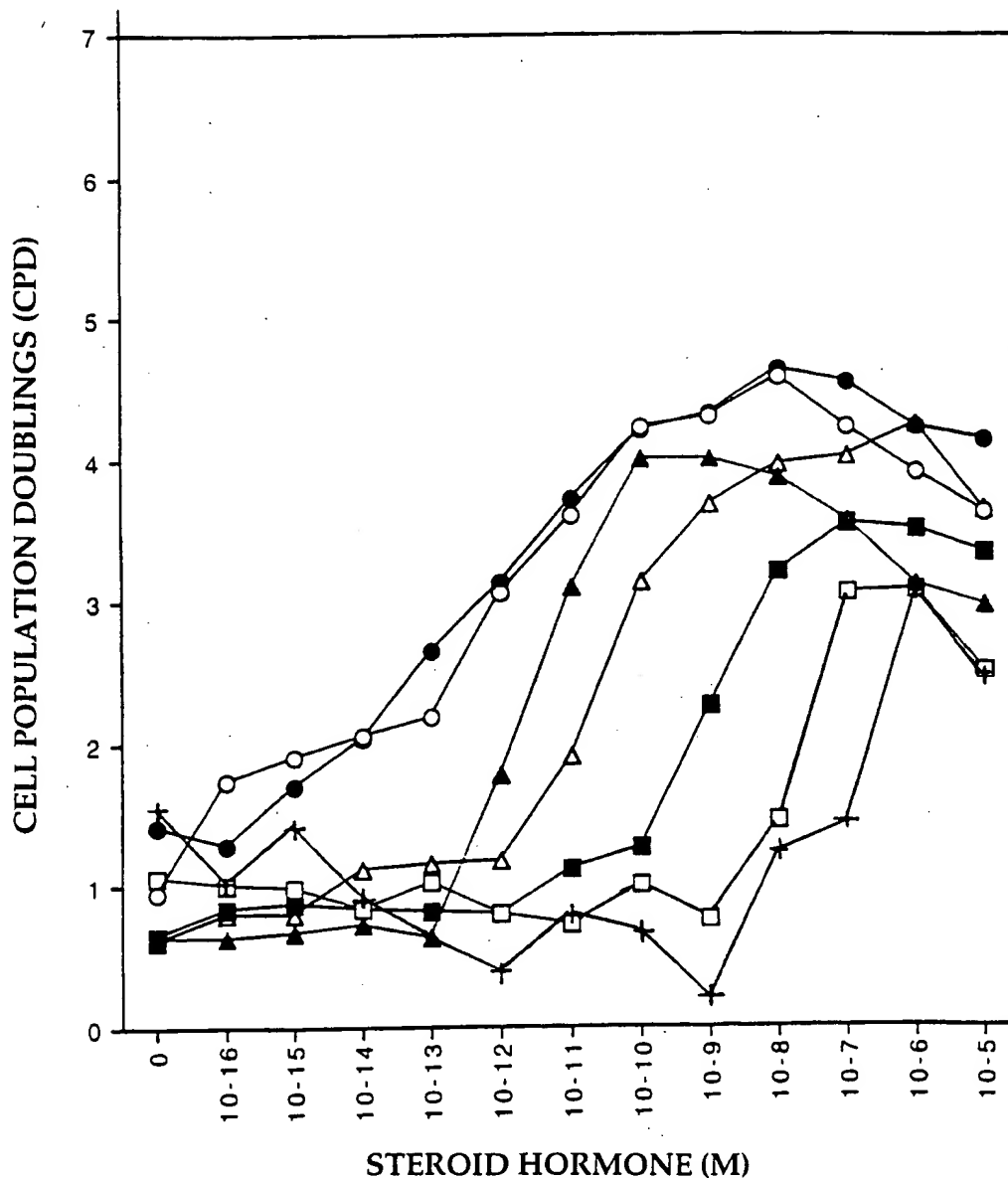
LEGEND:

Growth after 9 days is shown in response to:

- Closed circles = E₂
- Open circles = E₁
- Closed triangles = E₃
- Open triangles = DHT
- Closed squares = Testosterone
- Open squares = Progesterone
- Crosses = Cortisol

FIGURE 21

DOSE RESPONSE OF STEROID HORMONES
 WITH LNCaP CELLS IN 50% CDE - HORSE SERUM



LEGEND:

Growth after 14 days is shown in response to:

- Closed circles = E₂
- Open triangles = E₁
- Open squares = E₃
- Open circles = DHT
- Closed triangles = Testosterone
- Closed squares = Progesterone
- Crosses = Cortisol

FIGURE 22

**T₃ TITRATION OF GH₃ CELLS GROWN
IN SERUM - FREE MEDIUM (PCM)**

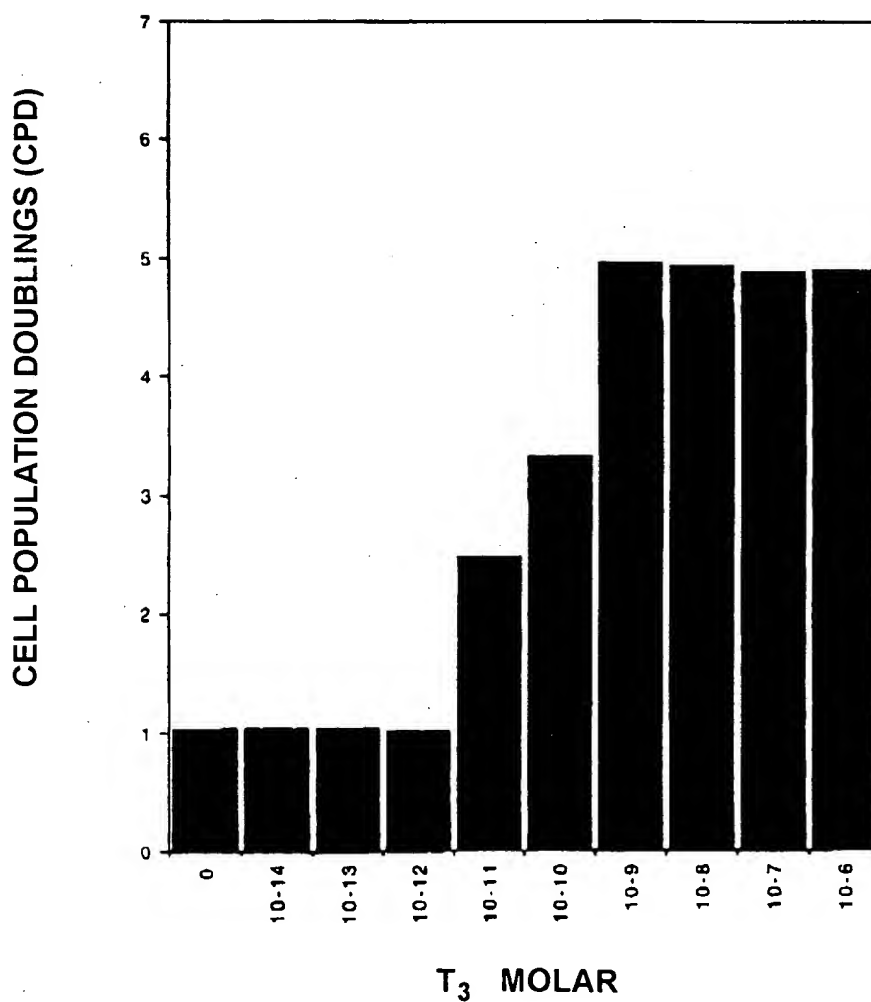


FIGURE 23

E_2 TITRATION OF GH₃ CELLS GROWN IN
SERUM-FREE MEDIUM MINUS T₃

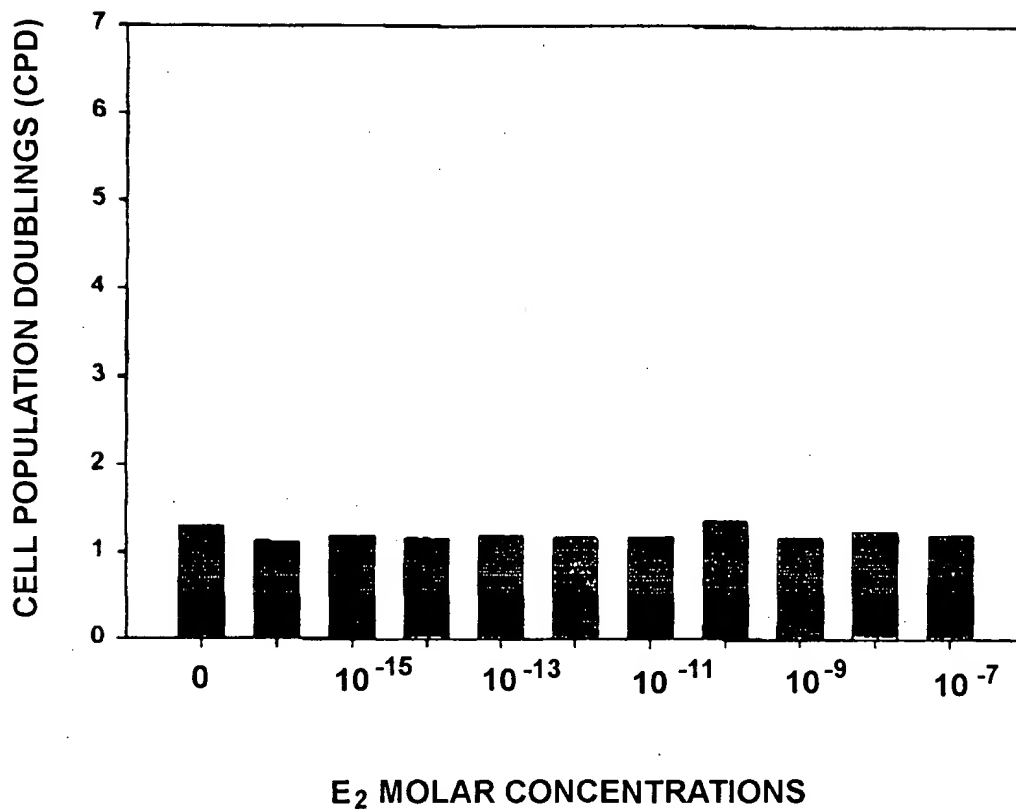


FIGURE 24

**EFFECT OF T₃ ON GH CELL LINES:
GROWTH IN 2.5% CDE - HORSE SERUM WITH NO E₂**

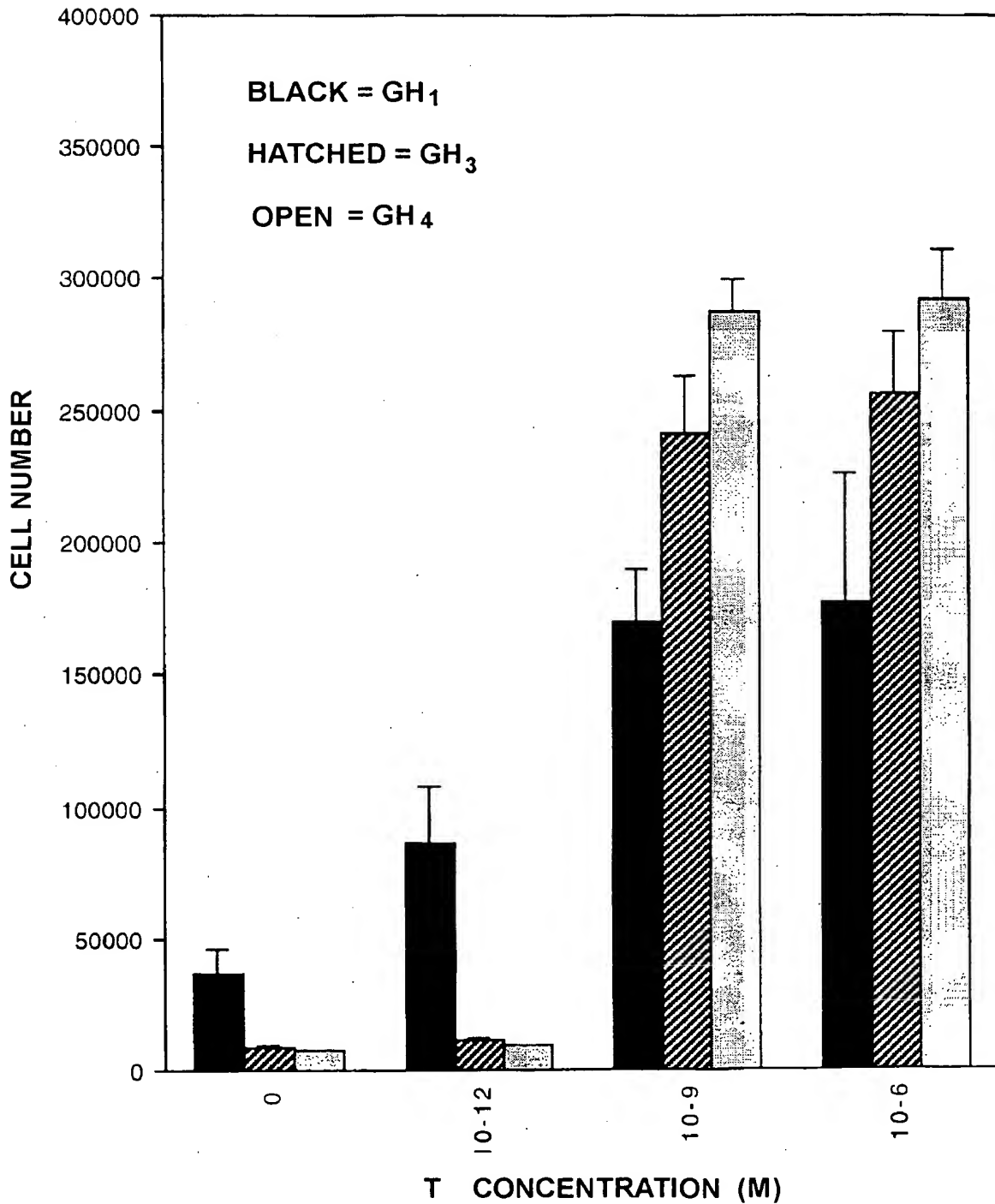


FIGURE 25

**EFFECT OF T_3 ON PITUITARY CELL LINES
INCUBATED IN 50% CDE - HORSE SERUM**

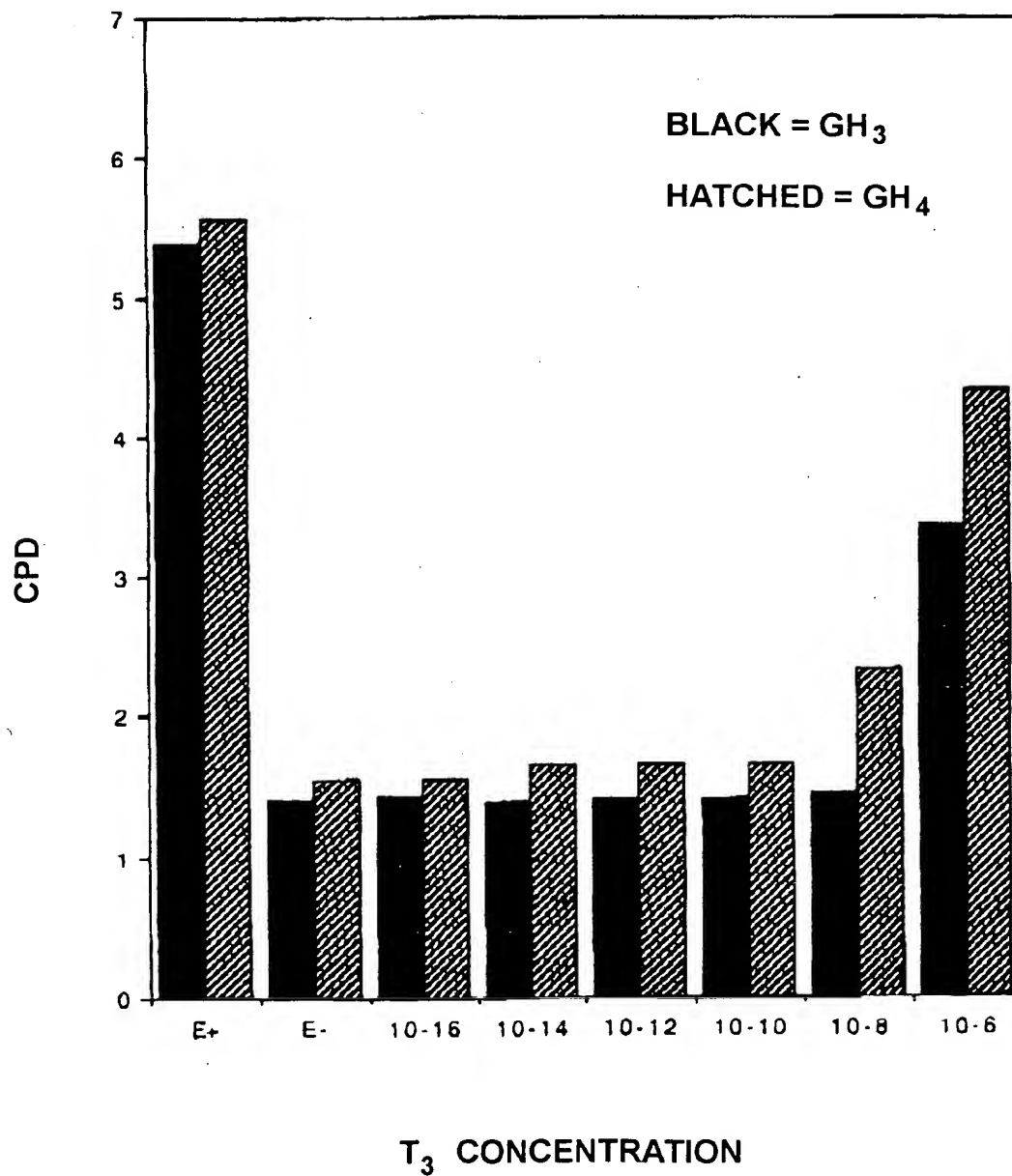
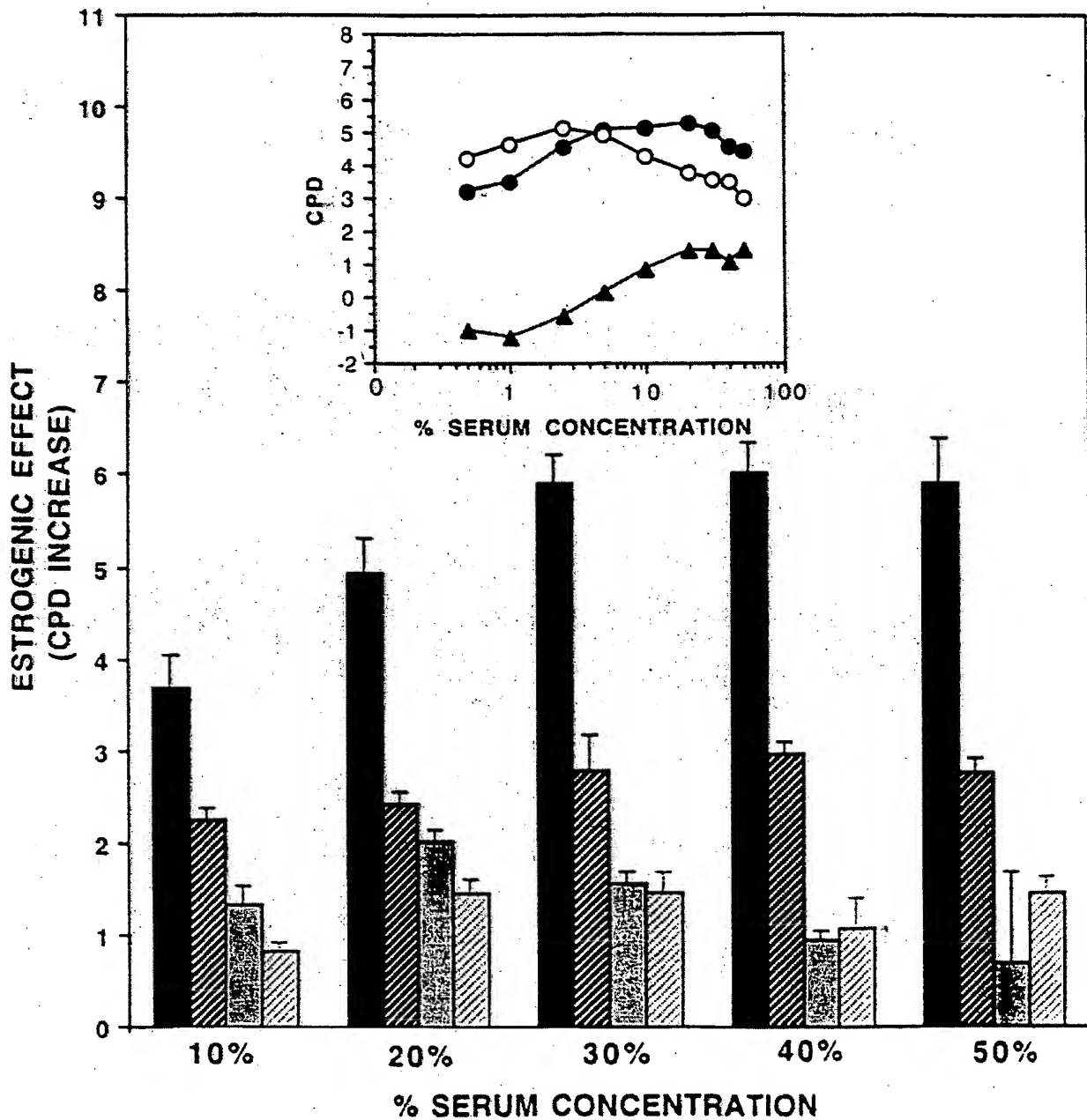


FIGURE 26

COMPARISON OF 56°C AND 34°C CHARCOAL EXTRACTED SERUM



FILLED BARS: Estrogenic effect in 34°C prepared CDE-serum

DARK HATCHED BARS: 56°C prepared CDE-serum

LIGHT SHADED BARS: Charcoal extracted at 34°C then charcoal extraction at 56°C

LIGHT HATCHED BARS: Charcoal extracted at 34°C then incubation for 20 min at 56°C

INSERT: Dose-response growth effects of horse serum extracted at 34°C followed by incubation for 20 min at 56°C

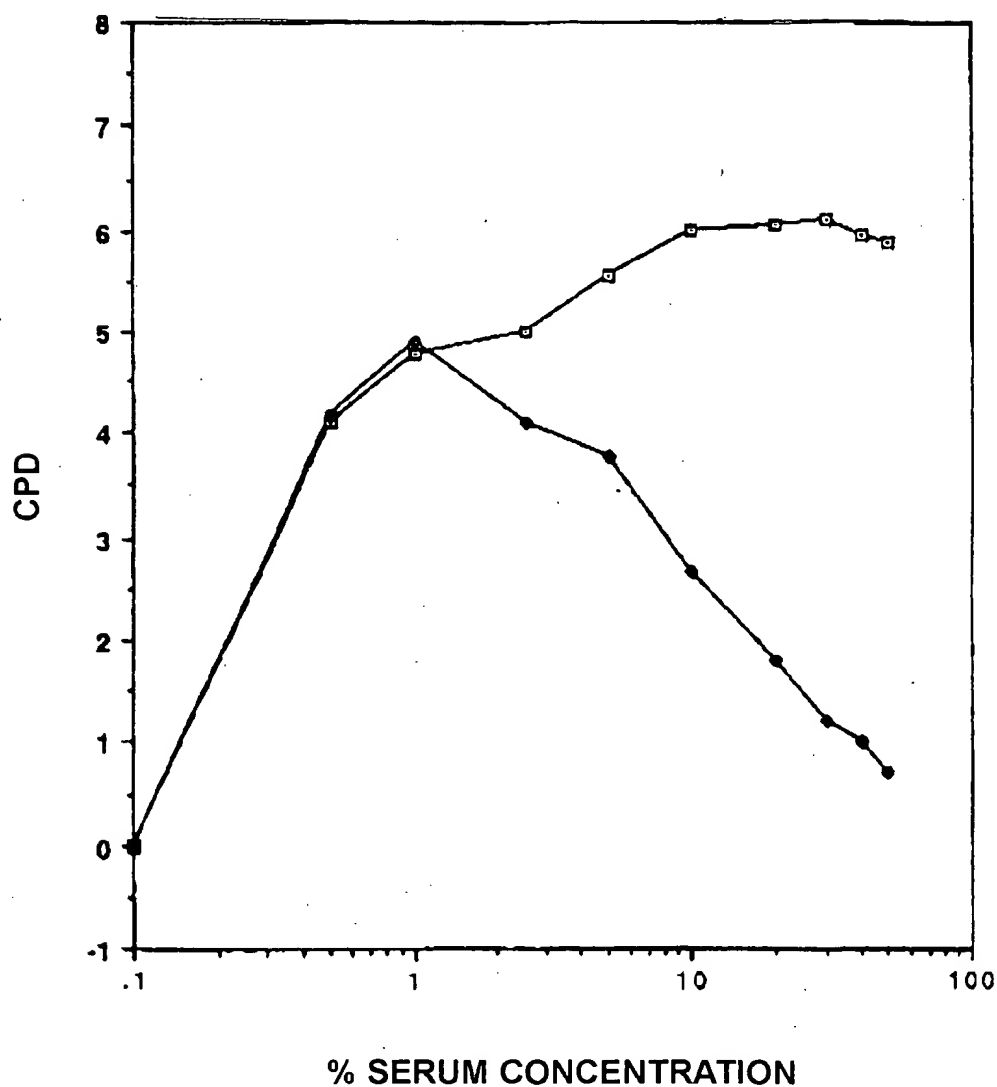
Open circles - Growth without E₂

Closed circles - Growth with 1.0 x 10⁻⁸ M E₂

Triangles - Estrogenic effect

FIGURE 27

HORSE SERUM TITRATION WITH MTW9/PL2 CELLS
EXTRACTION BY XAD-4 RESIN



LEGEND:

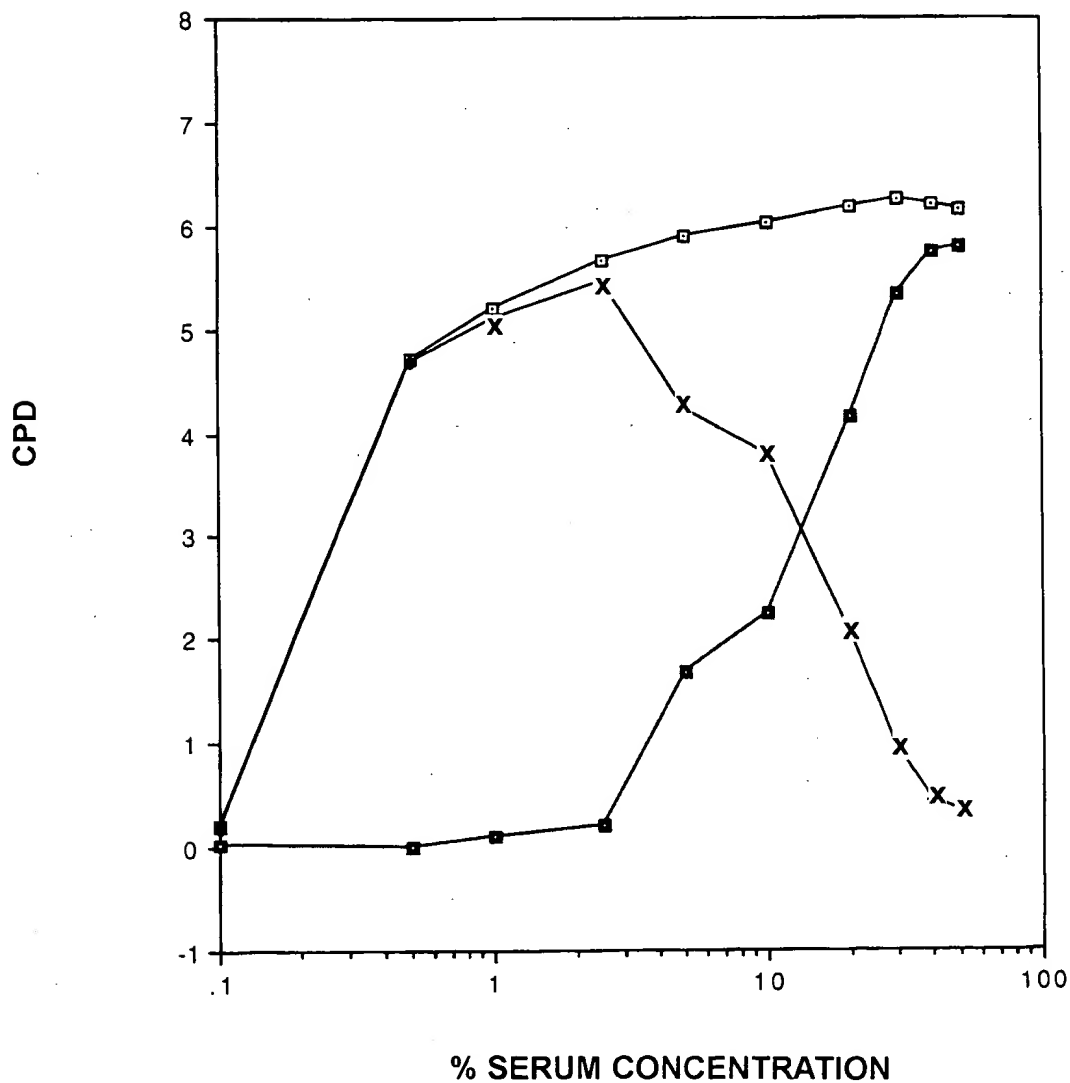
Open squares = + E₂

Closed squares = - E₂

FIGURE 28

HORSE SERUM TITRATION WITH T47D CELLS

EXTRACTION BY XAD-4 RESIN



LEGEND:

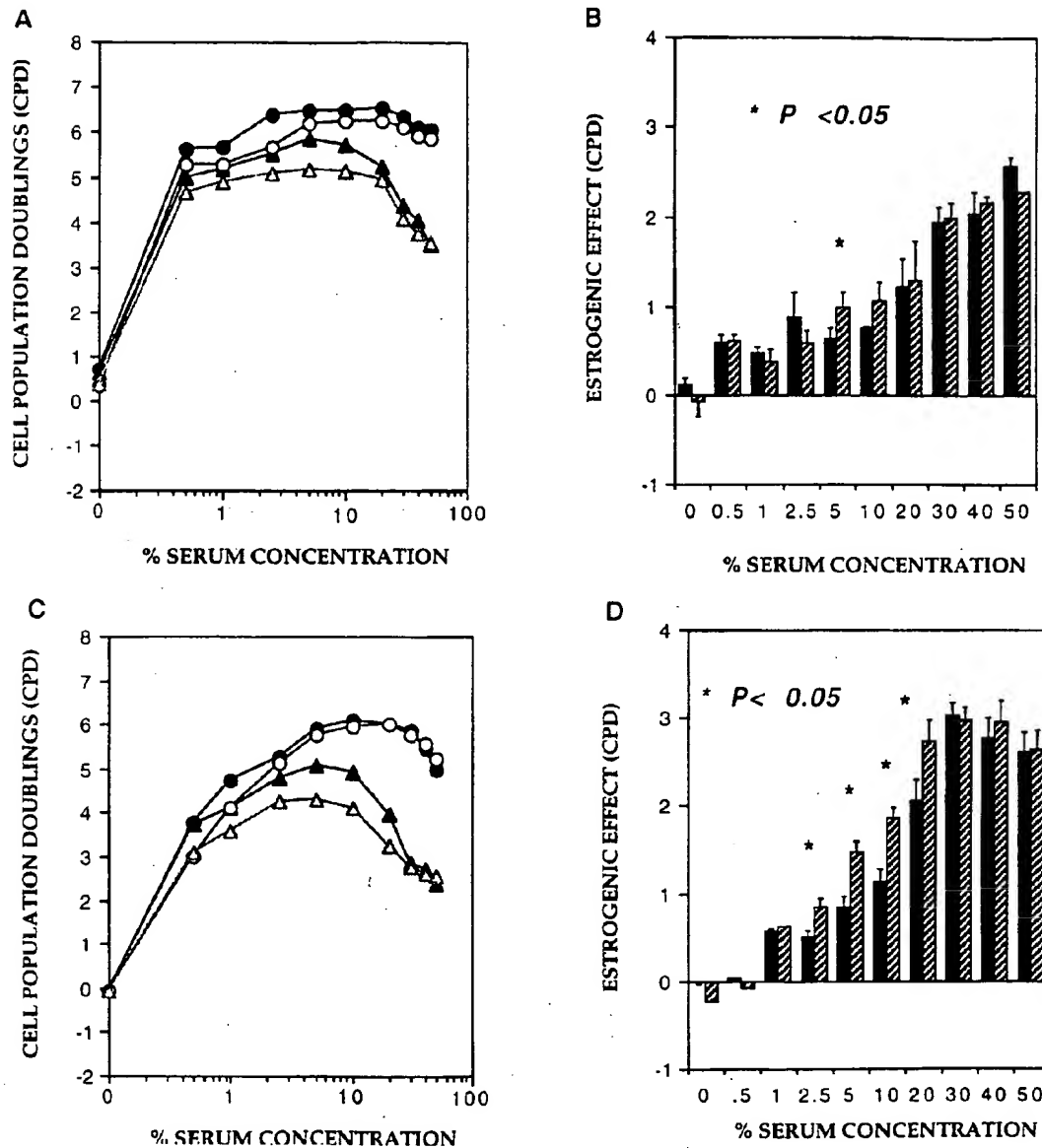
Open squares = + E₂

XXX = - E₂

Closed squares = Estrogenic effect

FIGURE 29

**MCF-7 CELL GROWTH IN CDE - HORSE SERUM
 ± PHENOL RED AND ± E₂**



LEGEND:

(A) MCF-7A cell growth in phenol red containing medium with E₂ (closed circles) and without E₂ (closed triangles), and in phenol red-free medium with E₂ (open circles) and without E₂ (open triangles).

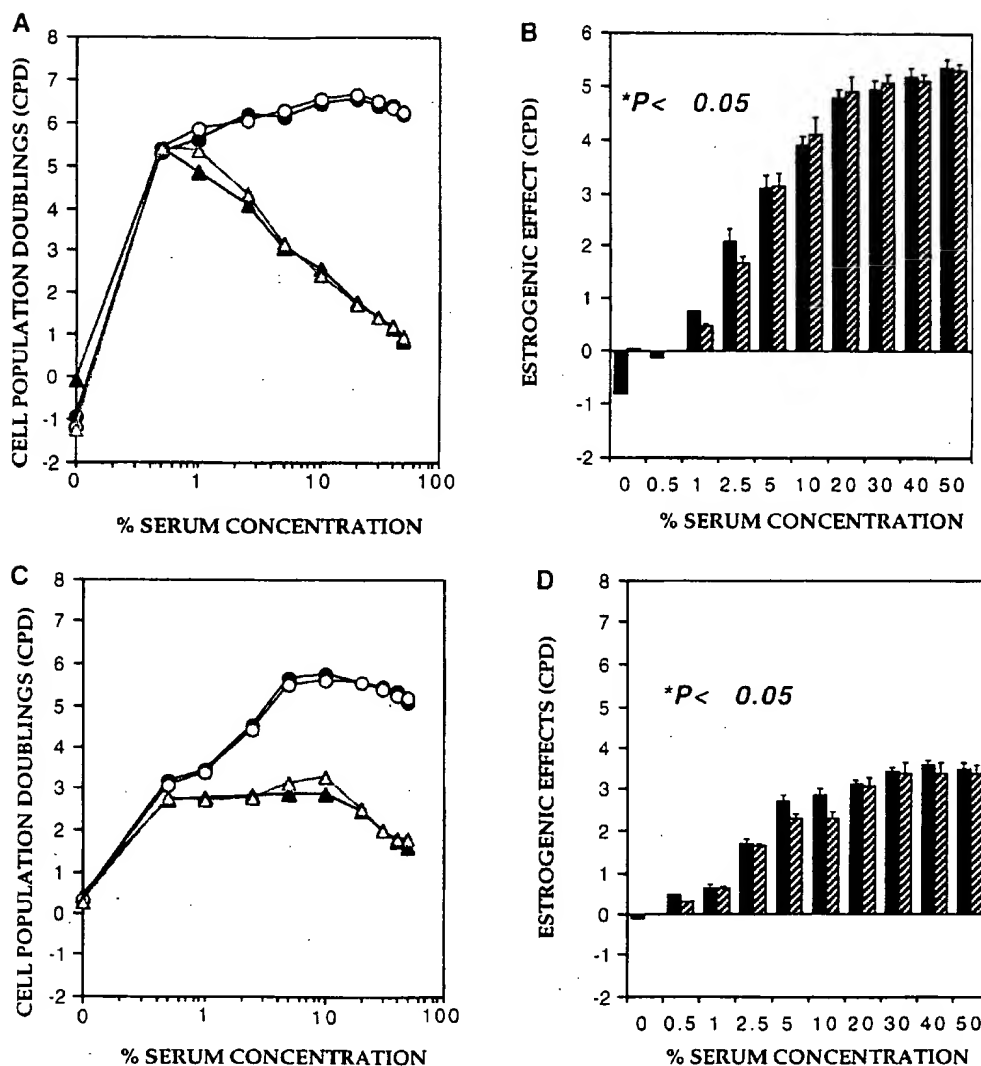
(B) Estrogenic effects with MCF-7A cells in medium with phenol red (solid bars) and without phenol red (shaded bars) were calculated from (A) and defined as the CPD in medium containing E₂ minus the CPD in medium without added E₂.

(C) MCF-7K cell growth in phenol red medium with E₂ (closed circles) and without E₂ (closed triangles), and in phenol red-free medium with E₂ (open circles) and without E₂ (open triangles).

(D) Estrogenic effects with MCF-7K cells in medium with phenol red (solid bars) and without phenol red (shaded bars), calculated from (C).

FIGURE 30

T47D AND ZR-75-1 CELL GROWTH
 IN CDE-HS \pm PHENOL RED AND \pm E₂

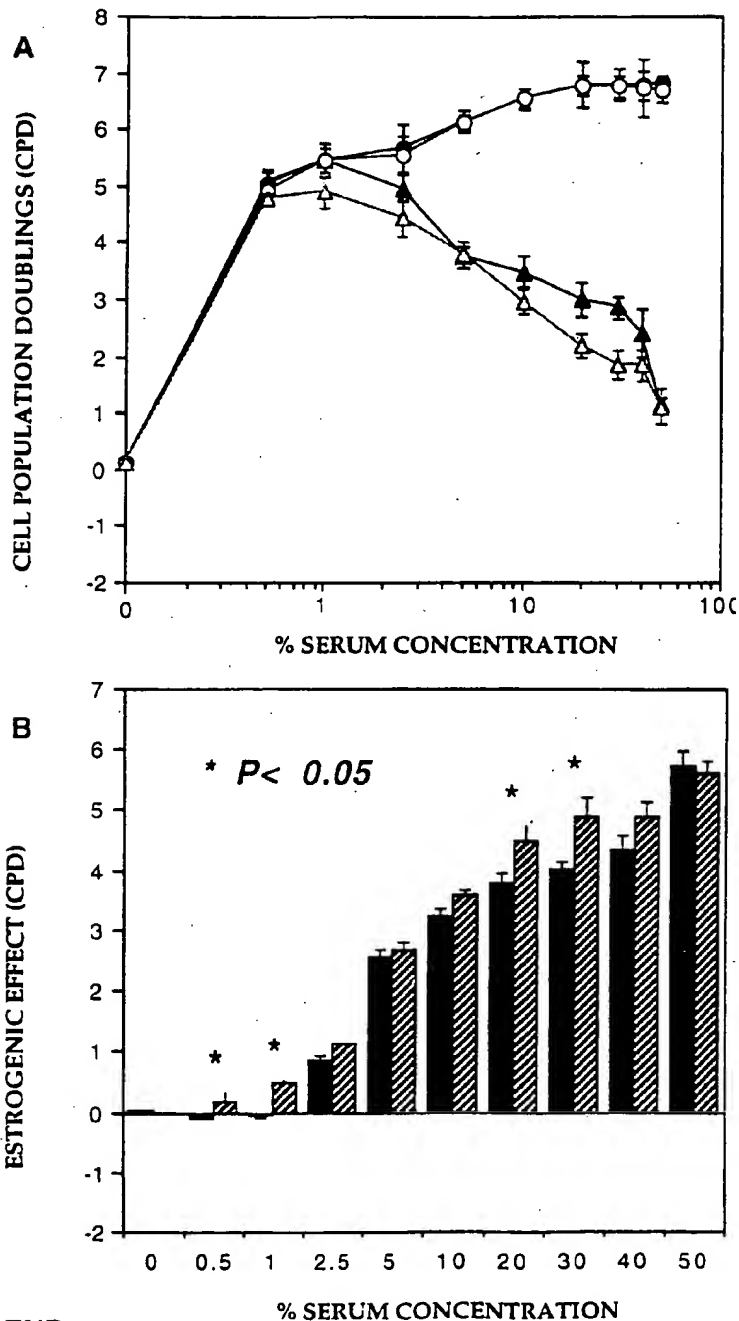


LEGEND:

- (A) T47D cell growth in phenol red containing medium with E₂ (closed circles) and without E₂ (closed triangles), and in phenol red-free medium with E₂ (open circles) and without E₂ (open triangles).
 (B) Estrogenic effects with T47D cells in medium with phenol red (solid bars) and without phenol red (shaded bars) were calculated from (A) and defined as the CPD in medium containing E₂ minus the CPD in medium without added E₂.
 (C) ZR-75-1 cell growth in phenol red medium with E₂ (closed circles) and without E₂ (closed triangles), and in phenol red-free medium with E₂ (open circles) and without E₂ (open triangles).
 (D) Estrogenic effects with ZR-75-1 cells in medium with phenol red (solid bars) and without phenol red (shaded bars), calculated from (C).

FIGURE 31

**MTW9/PL2 CELL GROWTH IN CDE - HORSE SERUM
 ± PHENOL RED AND ± E₂**



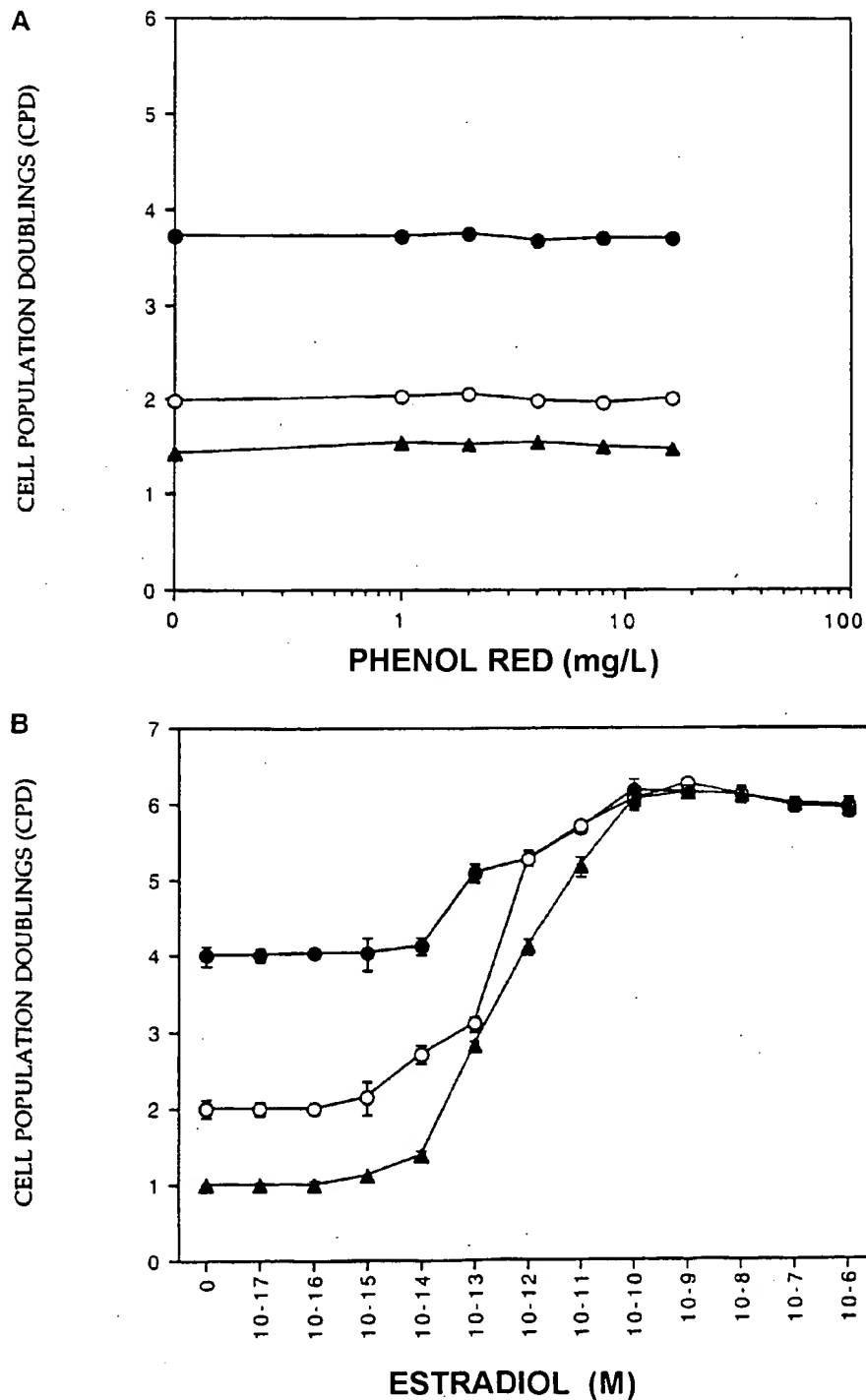
LEGEND:

(A) MTW9/PL2 growth in phenol red medium with E₂ (closed circles) and without E₂ (closed triangles), and in phenol red-free medium with E₂ (open circles) and without E₂ (open triangles).

(B) Estrogenic effects with MTW9/PL2 cells in medium with phenol red (solid bars) and without (shaded bars) were calculated from (A).

FIGURE 32

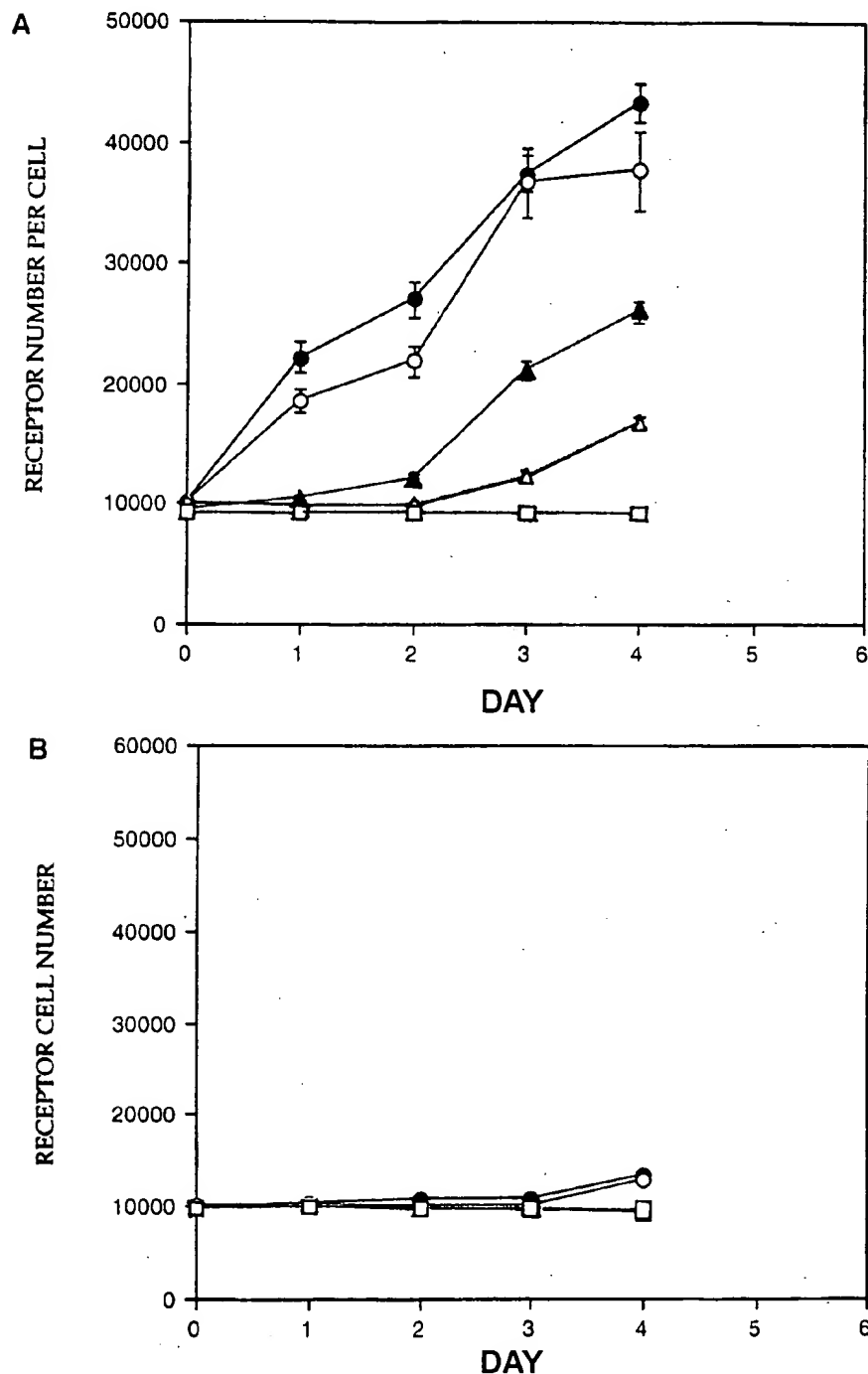
**DOSE RESPONSE TO PHENOL RED AND E₂
 IN THREE CELL LINES**



LEGEND: The growth of the MCF-7A (closed circles), MTW9/PL2 (open circles) and T47D (closed triangles) cell lines was assessed at 14, 7, and 12 days.

FIGURE 33

**PROGESTERONE RECEPTOR INDUCTION IN
 T47D CELLS BY PHENOL RED AND E₂**



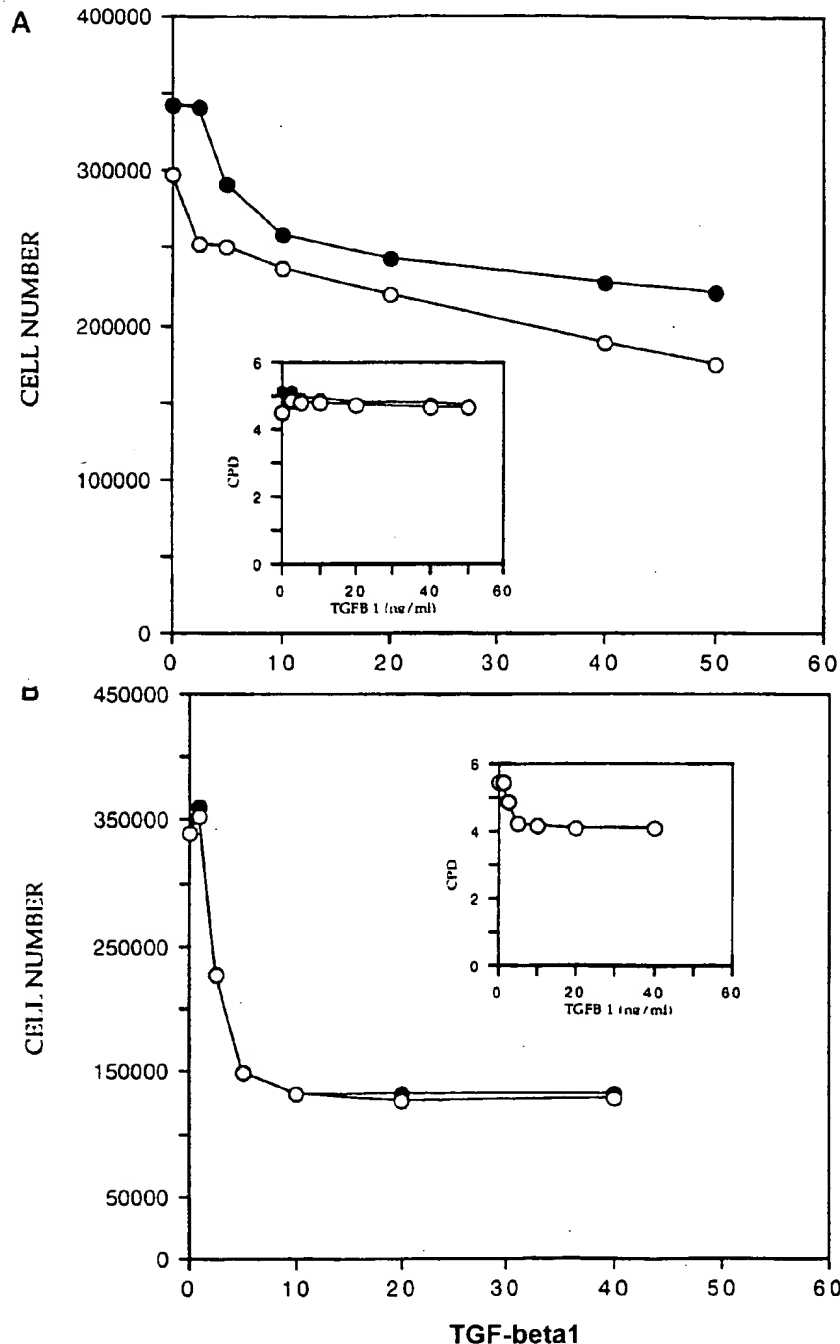
LEGEND:

(A) The effects of E₂ at 1.0 x 10⁻⁸ M (closed circles), 1.0 x 10⁻¹⁰ M (open circles), 1.0 x 10⁻¹² M (closed triangles), 1.0 x 10⁻¹⁴ M (open triangles) and the control without added E₂ (open squares).

(B) The effects of phenol red at 16 mg/L (closed circles), 8 mg/L (open circles), 4 mg/L (closed triangles), 2 mg/L (open triangles), and the control without phenol red (open squares).

FIGURE 34

EFFECT OF TGF-beta1 ON THE GROWTH OF BREAST/MAMMARY ORIGIN CELL LINES



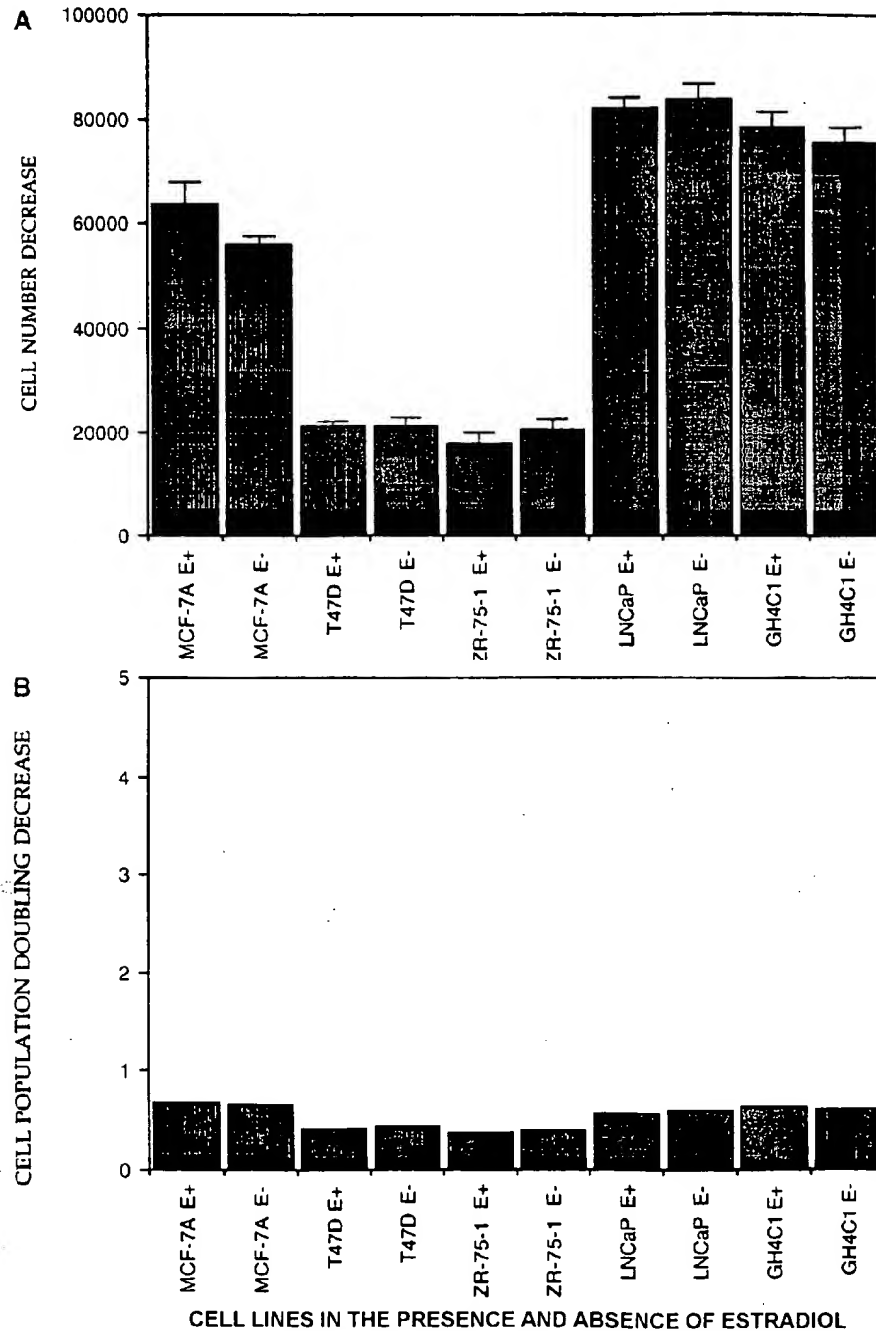
LEGEND:

(A) The effect of the transforming growth inhibitor on human breast MCF-7K cell growth as measured after 12 d either with 10 nM E₂ (closed circles) or without the hormone (open circles). The insert shows conversion of the cell number results to CPD.

(B) The same experiment with rat mammary MTW9/PL2 cells after 9 d growth.

FIGURE 35

EFFECT OF TGF-beta1 ON THE GROWTH OF CELL LINES FROM BOTH HUMAN AND RODENT TUMORS



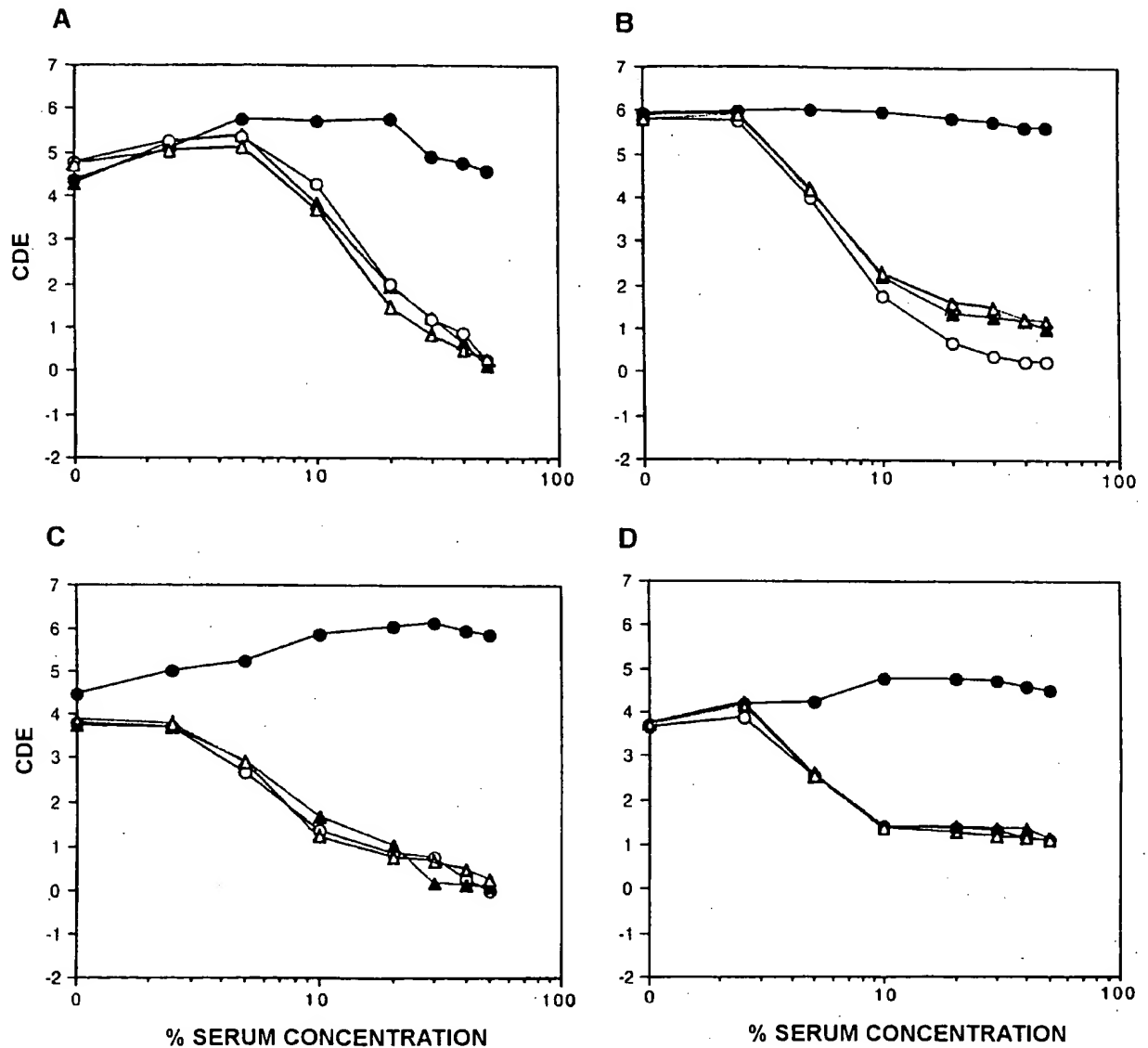
In these studies, TGF-beta1 was added at 40 ng/ml. Estradiol (\pm E) indicates either no added E_2 or the steroid at 10 nM.

(A) The effect of TGF-beta1 on five cell lines after 10-14 d growth in medium \pm E_2 . The results are expressed as cell number decreases caused by TGF-beta1.

(B) The CPD decreases caused by TGF-beta1 \pm E_2 with each of the cell lines shown in (A).

FIGURE 36

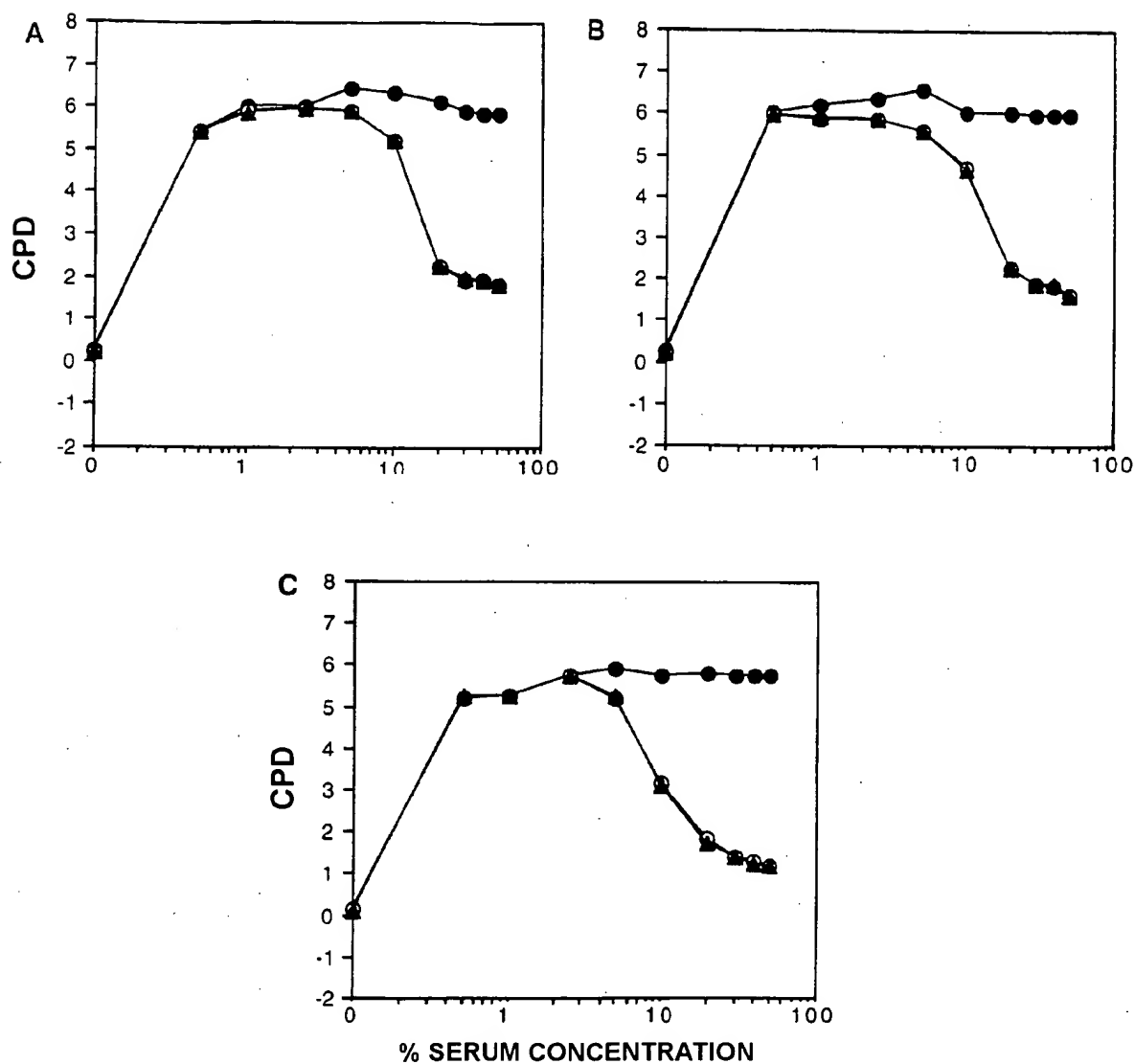
EFFECT OF EGF AND TGF-alpha ON THE GROWTH
 OF HUMAN BREAST CANCER CELLS



The cells were grown in D-MEM/F-12 supplemented with increasing concentrations of CDE horse serum. Each line tested was grown in serum alone (open circles) and in serum plus 50 ng/ml EGF (open triangles), 50 ng/ml TGF-alpha (closed triangles), or 10 nM E₂ without exogenous growth factors (closed circles). (A) - (D) show the results with the MCF-7A, MCF-7K, T47D, and ZR-75-1 cell lines, respectively.

FIGURE 37

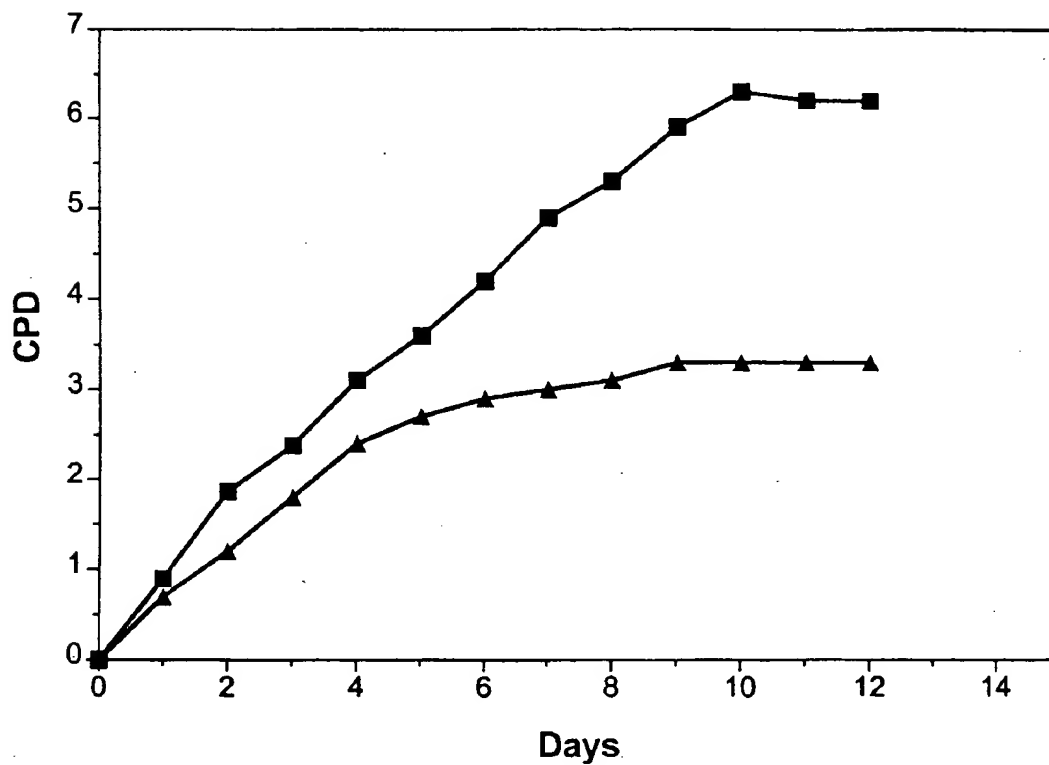
EFFECT OF IGF-I ON THE GROWTH OF HUMAN BREAST CANCER CELLS



Breast cancer cells were grown in D-MEM/F-12 supplemented with increasing concentrations of CDE horse serum. Each cell line tested was grown in serum alone (open circles) and in serum plus 1.0 ug/ml IGF-I (triangles), or in serum with 10 nM E₂ without exogenous growth factors (closed circles). (A) - (C) show the results with the MCF-7K, MCF-7A and T47D cells, respectively. Assays were conducted for 12-14 d.

FIGURE 38

T47D CELLS IN STANDARD D-MEM/F-12 MEDIUM
VS "LOW FE" SERUM-FREE SERUM

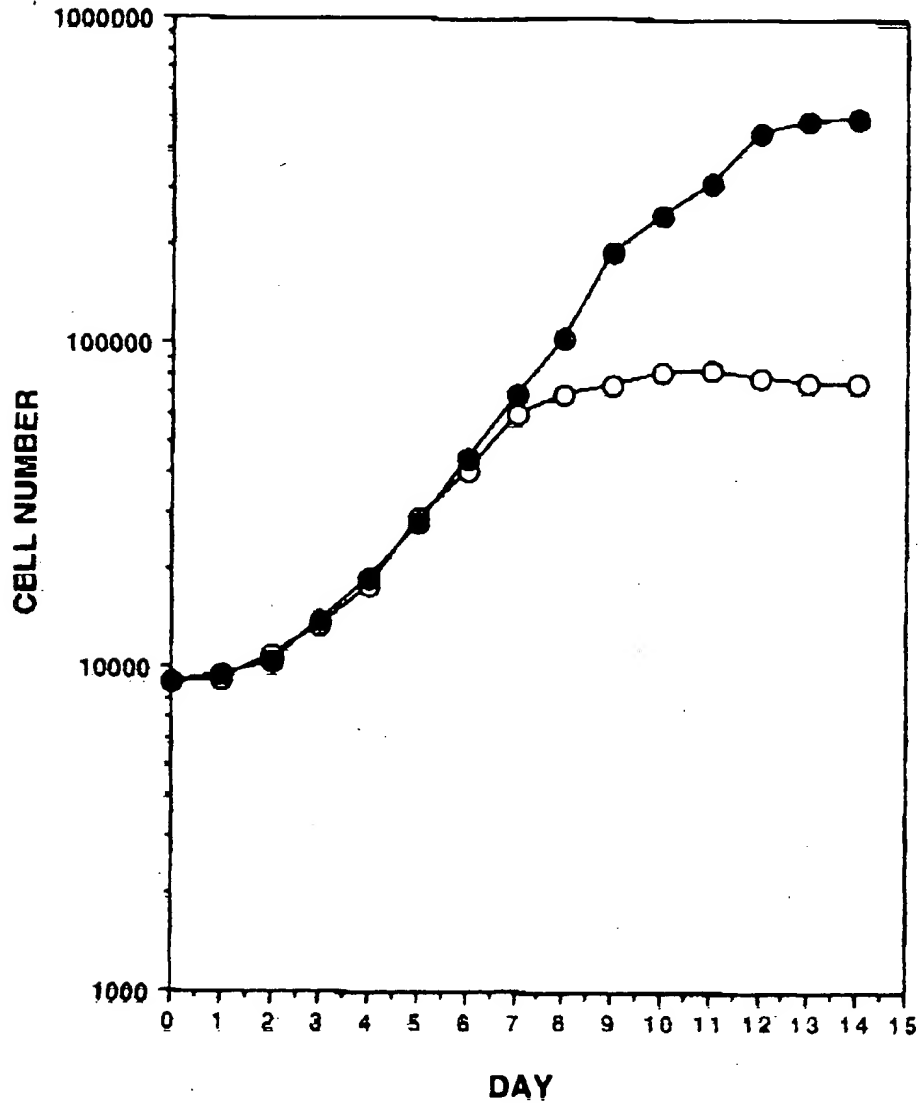


LEGEND:

- "STANDARD" MEDIUM
- ▲— "LOW-FE" MEDIUM

FIGURE 39

**LNCaP CELLS IN STANDARD D-MEM/F-12 MEDIUM
VS "LOW-FE" SERUM-FREE MEDIUM**

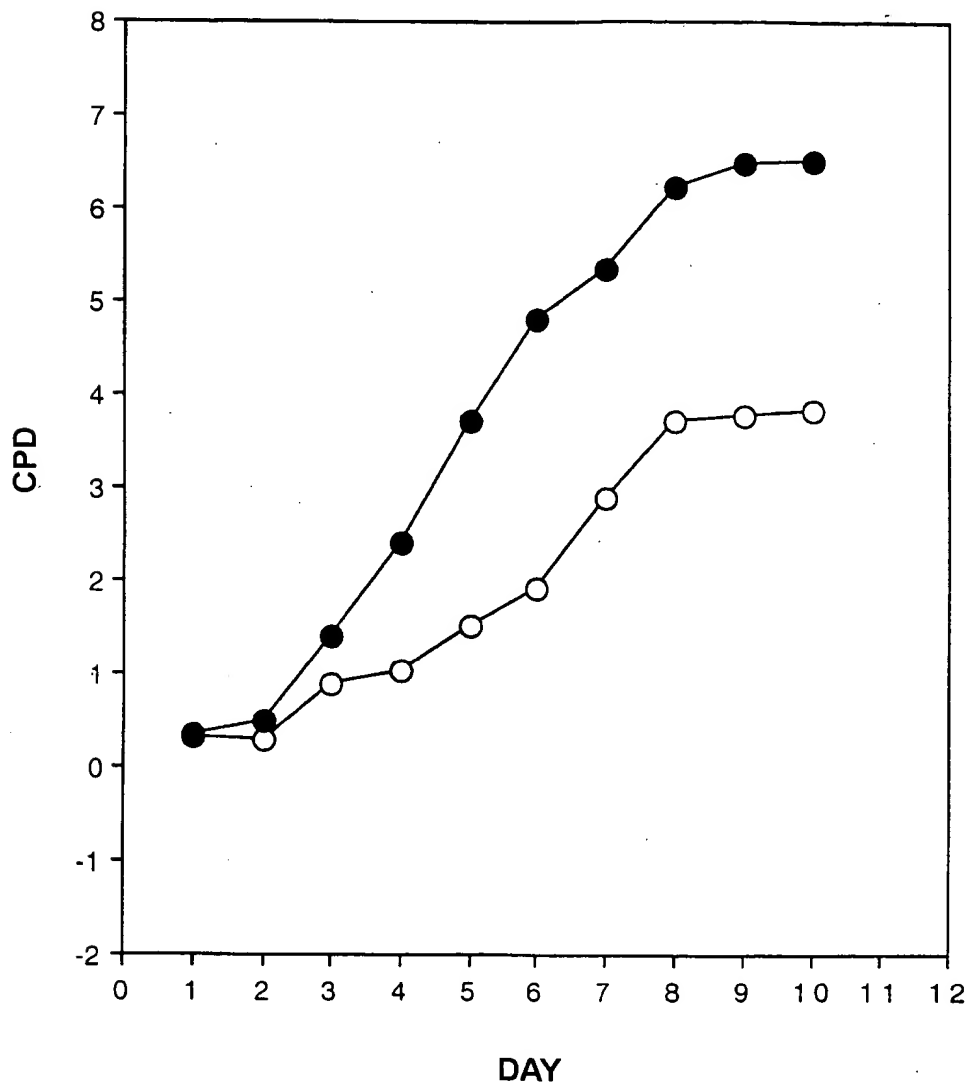


LEGEND:

- "STANDARD" MEDIUM
- "LOW-FE" MEDIUM

FIGURE 40

**MDCK CELLS IN STANDARD D-MEM/F-12 MEDIUM
VS "LOW FE" SERUM-FREE MEDIUM**

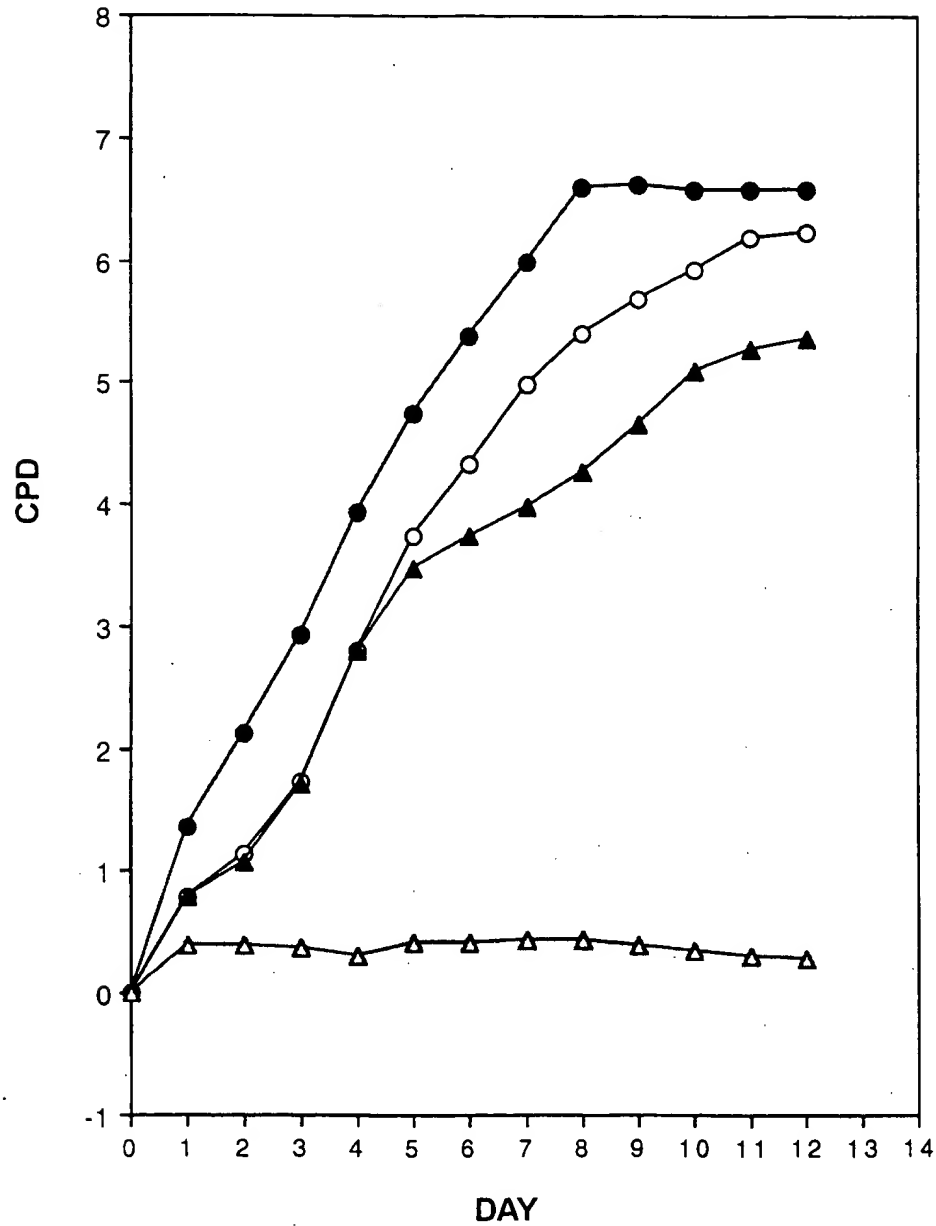


LEGEND:

- "STANDARD" MEDIUM
- "LOW-FE" MEDIUM

FIGURE 41

**LNCaP CELL GROWTH IN CAPM \pm DHT
AND 10% FETAL BOVINE SERUM**

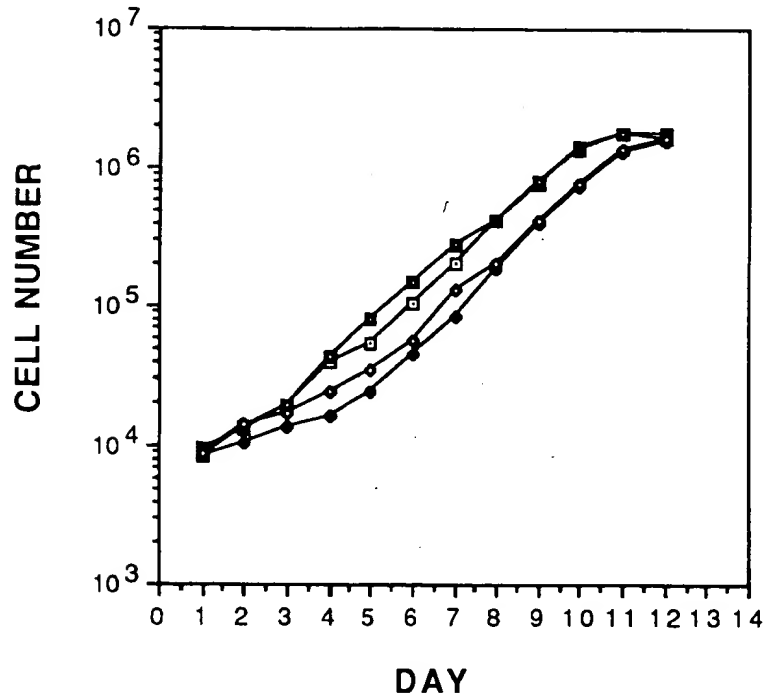


LEGEND:

Closed circles = Fetal bovine serum
Open circles = CAPM + DHT
Closed triangles = CAPM - DHT
Open triangles = D-MEM/F12 only

FIGURE 42

**PC3 AND DU145 GROWTH IN SERUM - FREE
MEDIUM VS MEDIUM WITH 10% FETAL CALF SERUM**



LEGEND:

- PC3 IN SERUM-FREE MEDIUM
- ◆— DU145 IN SERUM-FREE MEDIUM
- PC3 IN 10% FETAL CALF SERUM
- DU145 IN 10% FETAL CALF SERUM

FIGURE 43

**DOSE RESPONSE EFFECTS OF CAPM
 SERUM - FREE MEDIUM COMPONENTS**

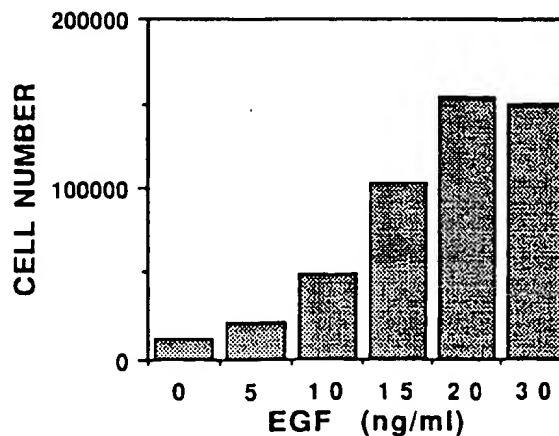
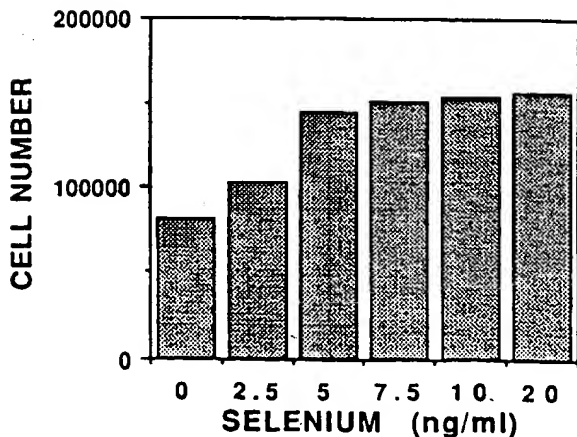
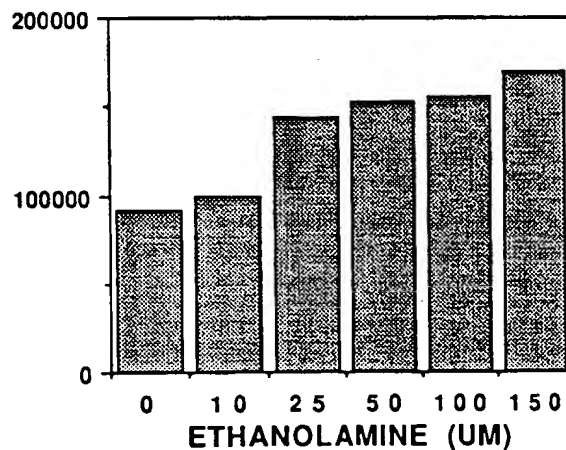
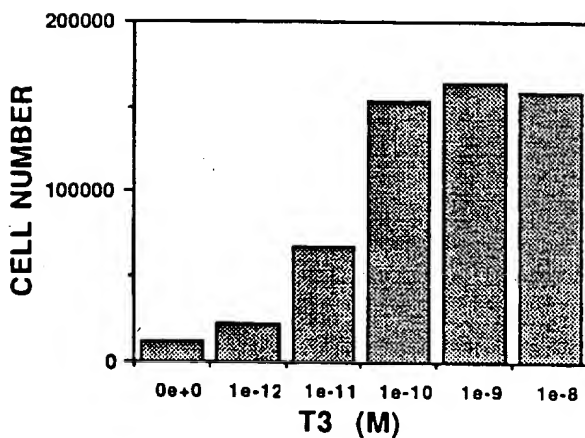
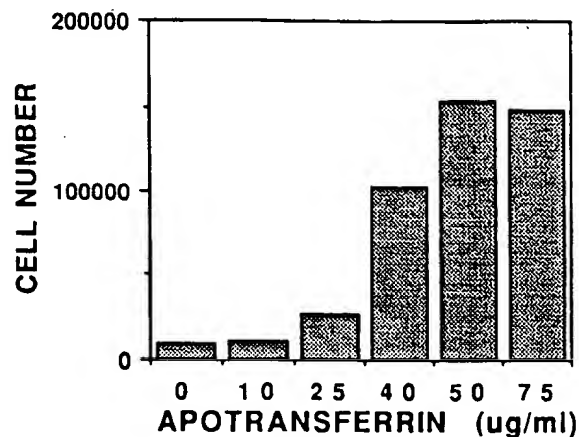
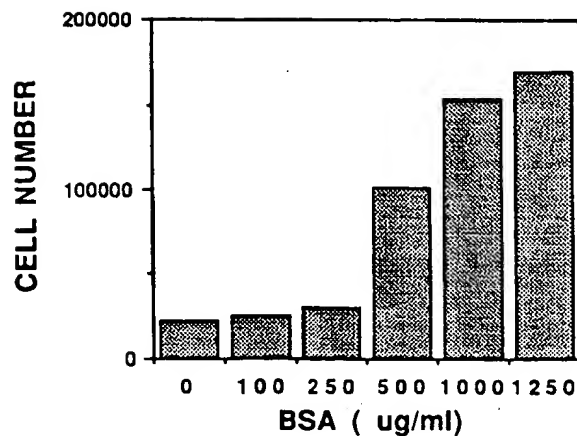
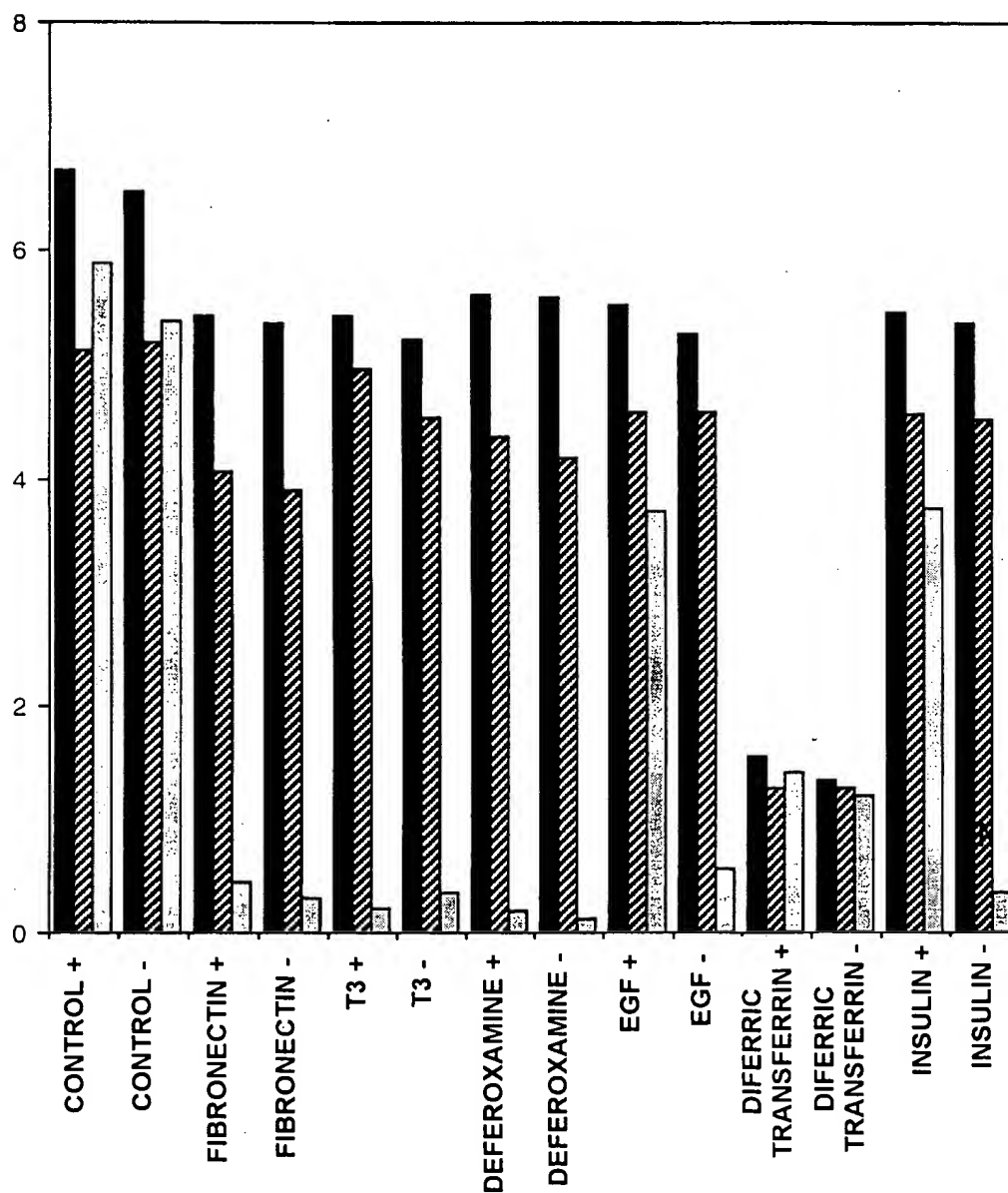


FIGURE 43

FIGURE 44

DELETIONS OF INDIVIDUAL COMPONENTS
 OF CAPM WITH PROSTATE CANCER CELL LINES



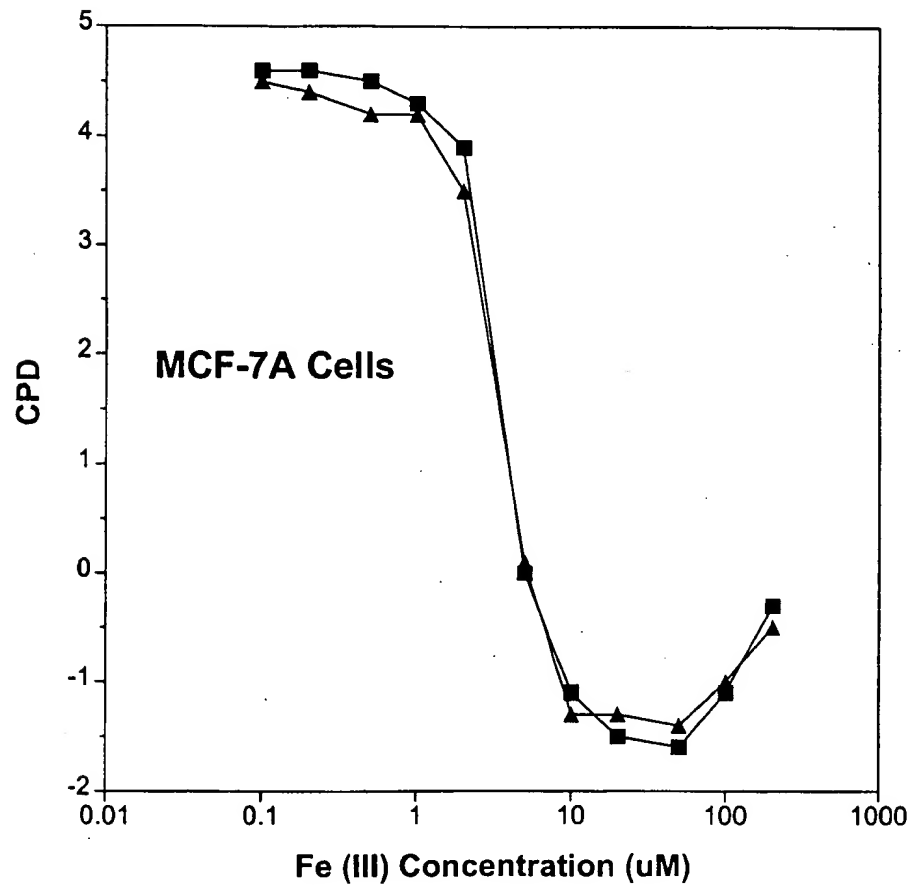
LEGEND:

- = PC3
- ▨ = DU145
- = LNCaP
- + = 10 nM DHT
- = NO DHT

FIGURE 44

FIGURE 45

**EFFECT OF FE (III) IN MCF-7A CELL GROWTH
IN DDM-2MF DEFINED MEDIUM**

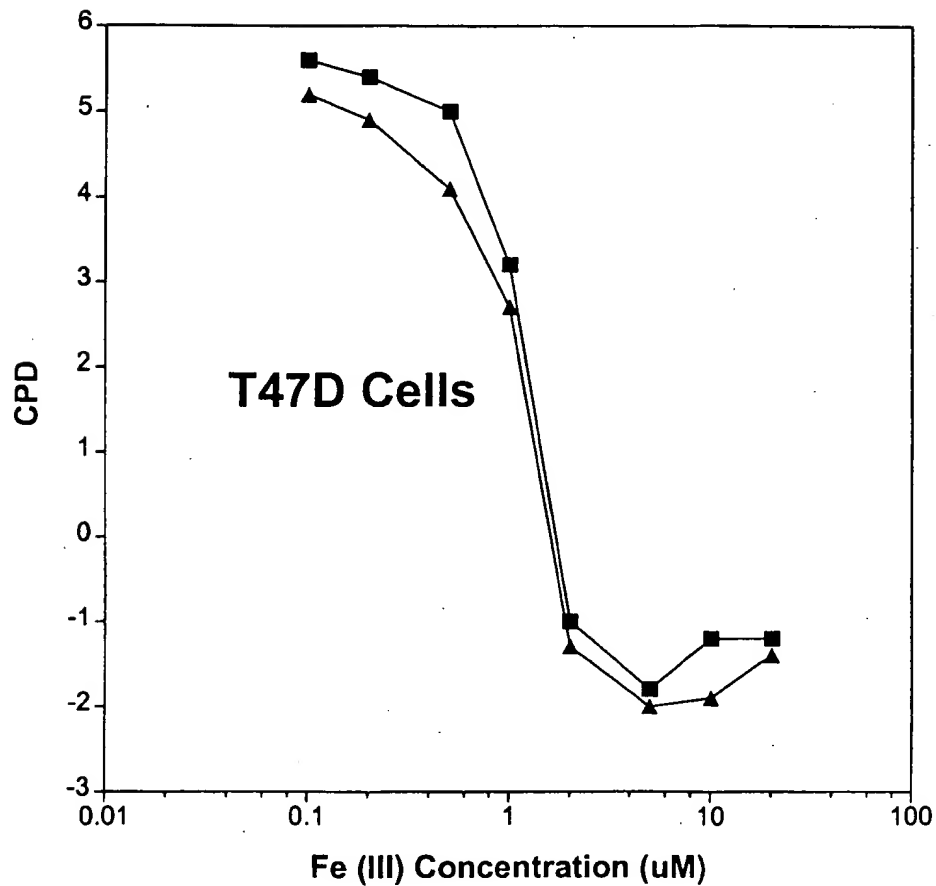


LEGEND:

- plus E₂
- ▲— minus E₂

FIGURE 46

EFFECT OF FE (III) IN T47D CELL GROWTH
IN DDM-2MF DEFINED MEDIUM

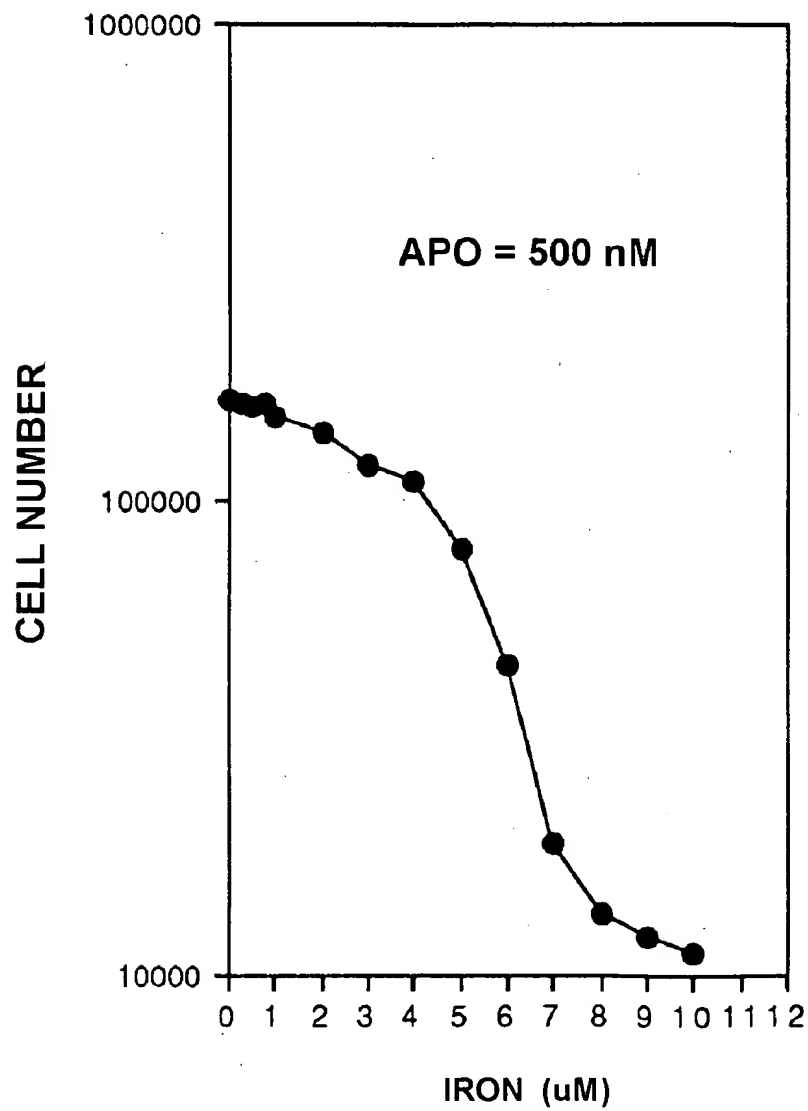


LEGEND:

- plus E₂
- ▲— minus E₂

FIGURE 47

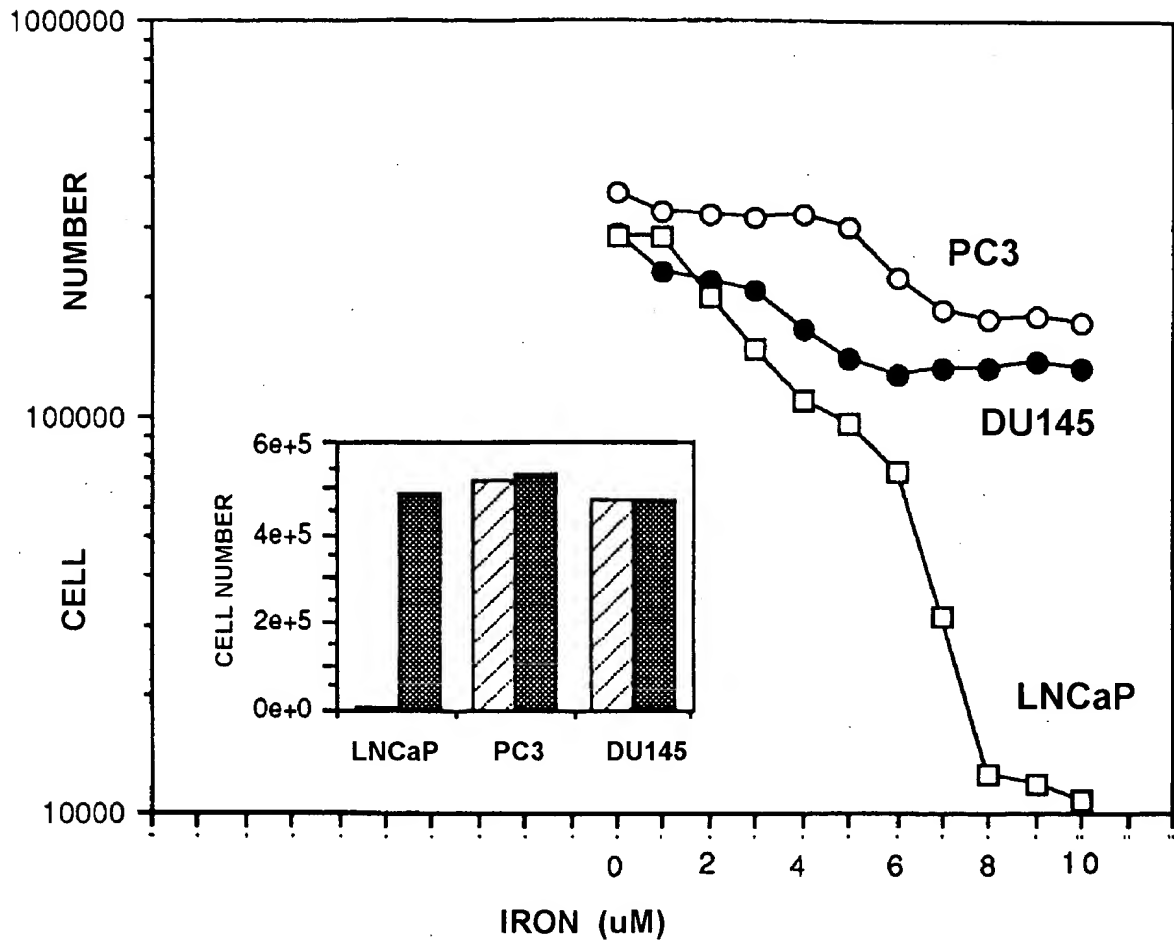
**EFFECTS OF INCREASING CONCENTRATIONS OF
IRON ON LNCaP CELLS GROWN IN SERUM-FREE
MEDIUM WITH APOTRANSFERRIN**



TOP SECRET

FIGURE 48

EFFECTS OF IRON AND T_3 ON THREE PROSTATIC CELL LINES IN SERUM-FREE MEDIUM



INSERT:

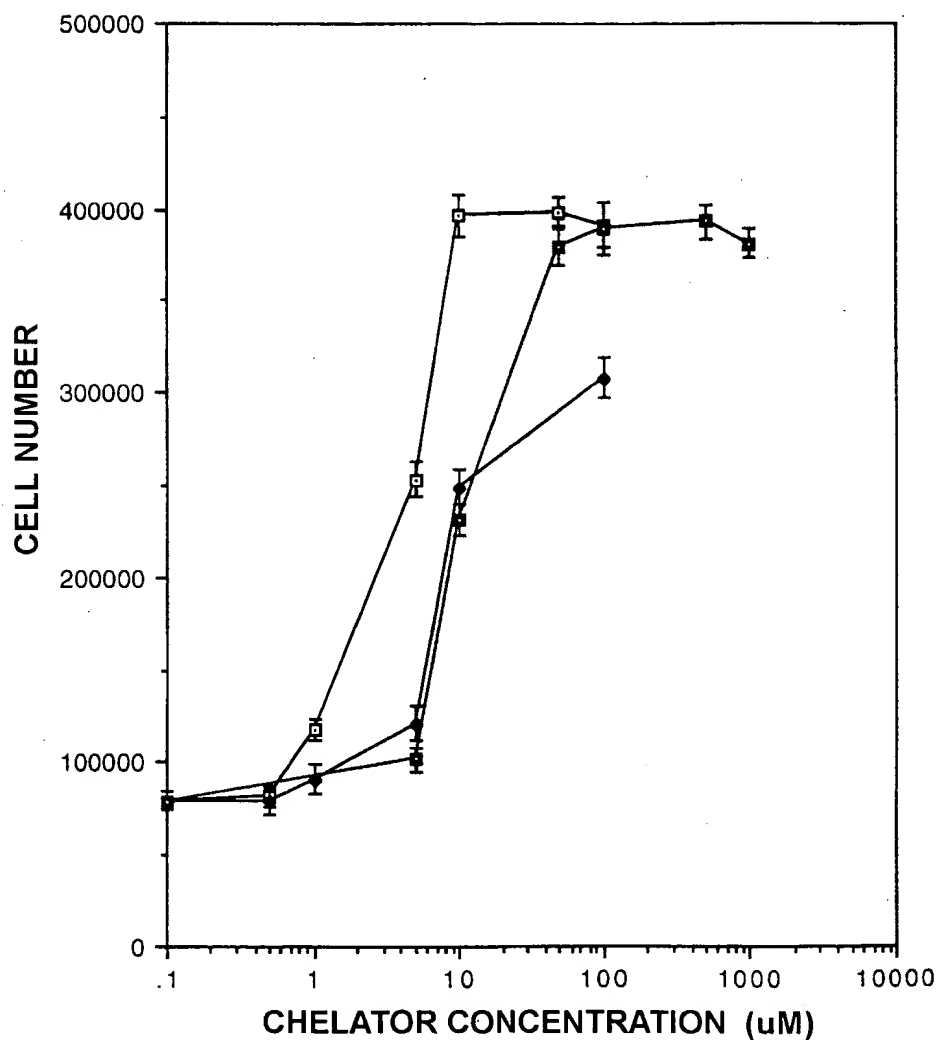
DARK BARS = GROWTH IN CAPM PLUS T_3

LIGHT (HATCHED) BARS = GROWTH IN CAPM MINUS T_3

NOTE THE STRIKING DEPENDENCE OF LNCaP CELLS ON T_3

FIGURE 49

EFFECT OF CHELATORS ON SERUM-FREE T47D
GROWTH UNDER HIGH IRON CONDITIONS

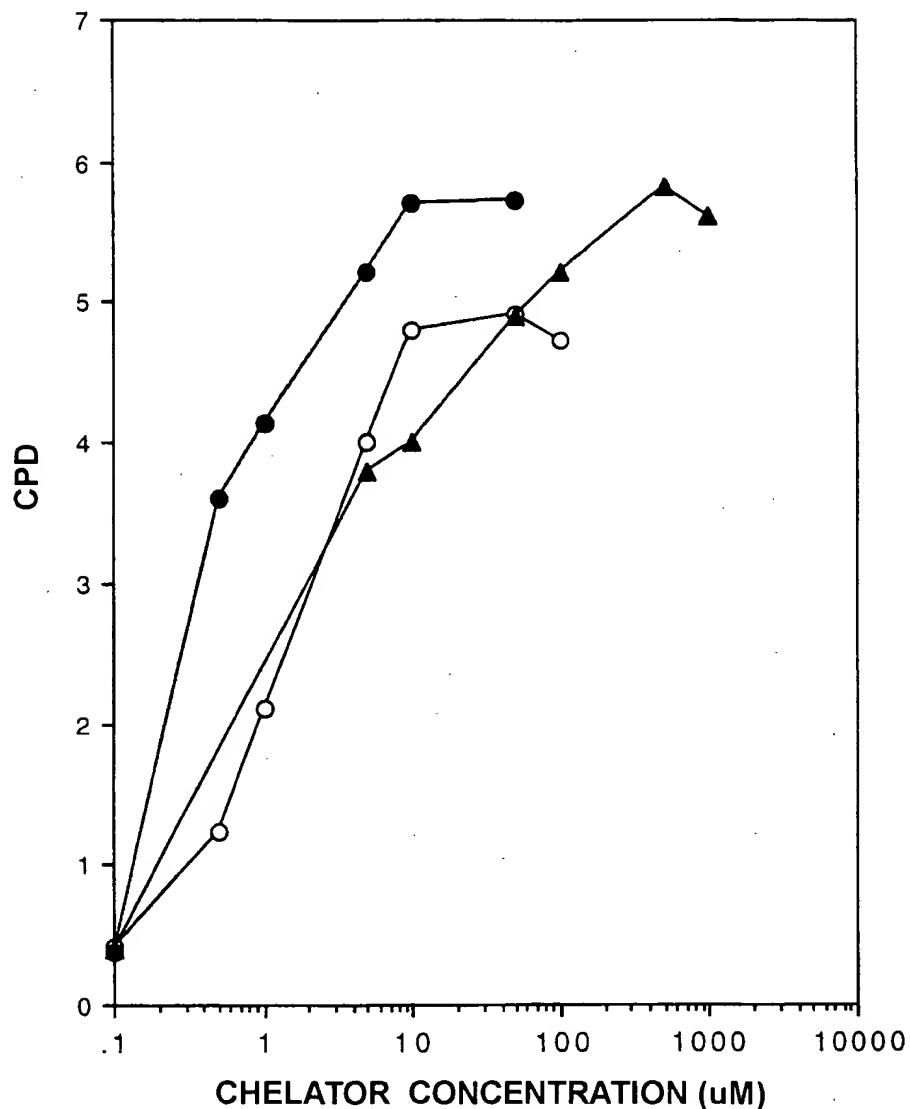


LEGEND:

- DEFEROXAMINE
- ♦— EDTA
- CITRATE

FIGURE 50

**EFFECT OF CHELATORS ON SERUM-FREE LNCaP
GROWTH UNDER HIGH IRON CONDITIONS**



LEGEND:

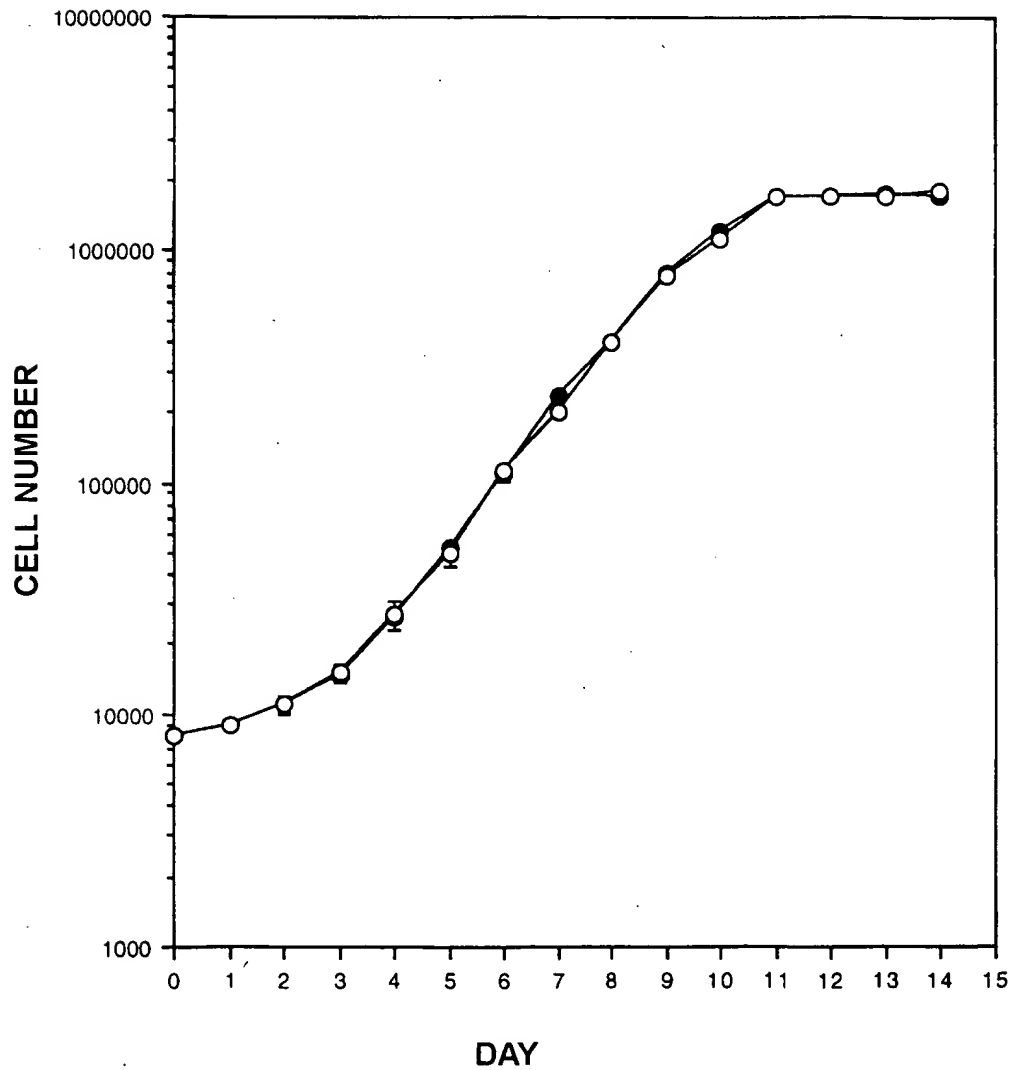
Closed circles = Deferoxamine

Open circles = Citrate

Closed triangles = EDTA

FIGURE 51

**DU145 GROWTH IN SERUM-FREE MEDIUM
BASED ON "LOW FE" OR "STANDARD" MEDIUM**



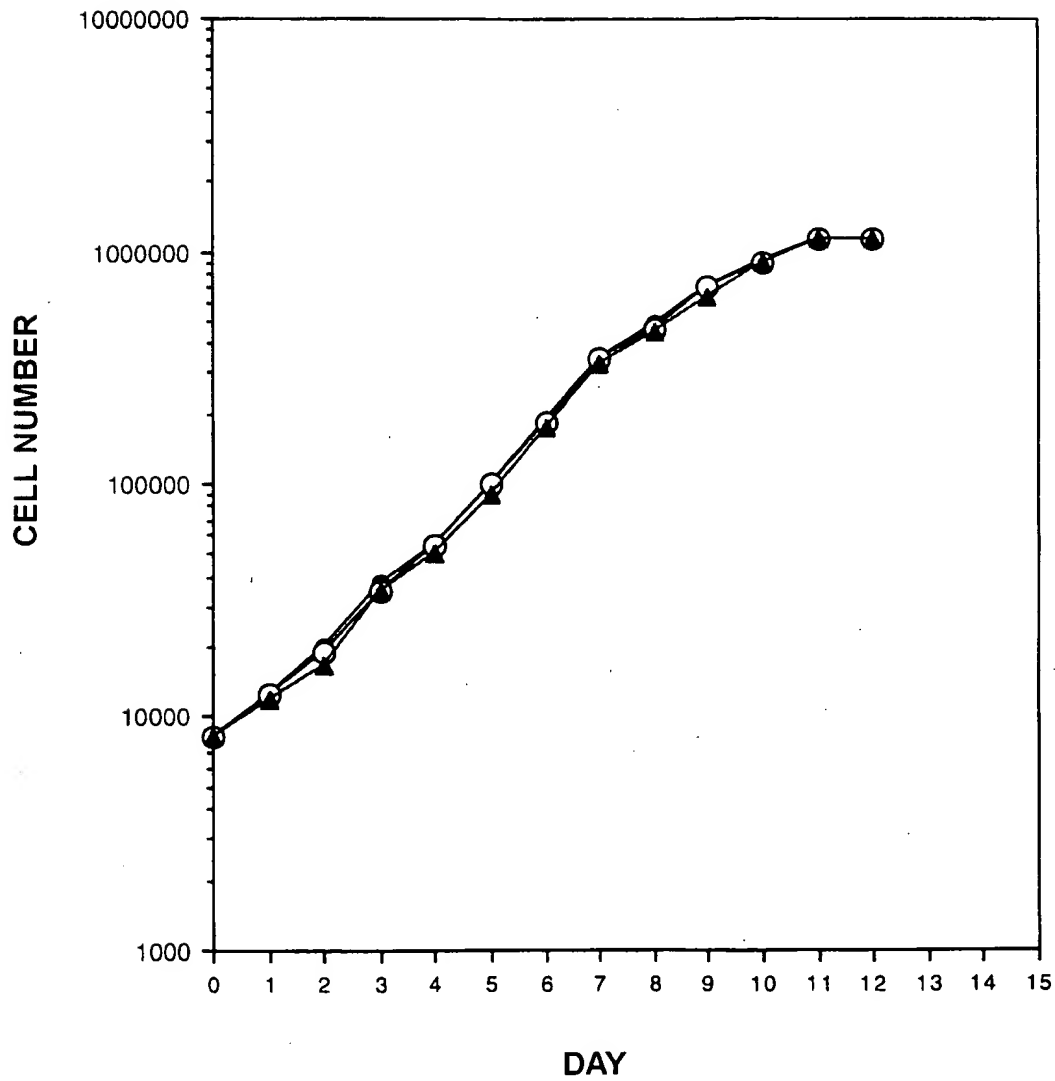
LEGEND:

Open circles = "Low Fe" medium

Closed circles = "Standard" medium

FIGURE 52

PC3 GROWTH IN SERUM-FREE MEDIUM BASED ON "LOW FE" OR "STANDARD" MEDIUM



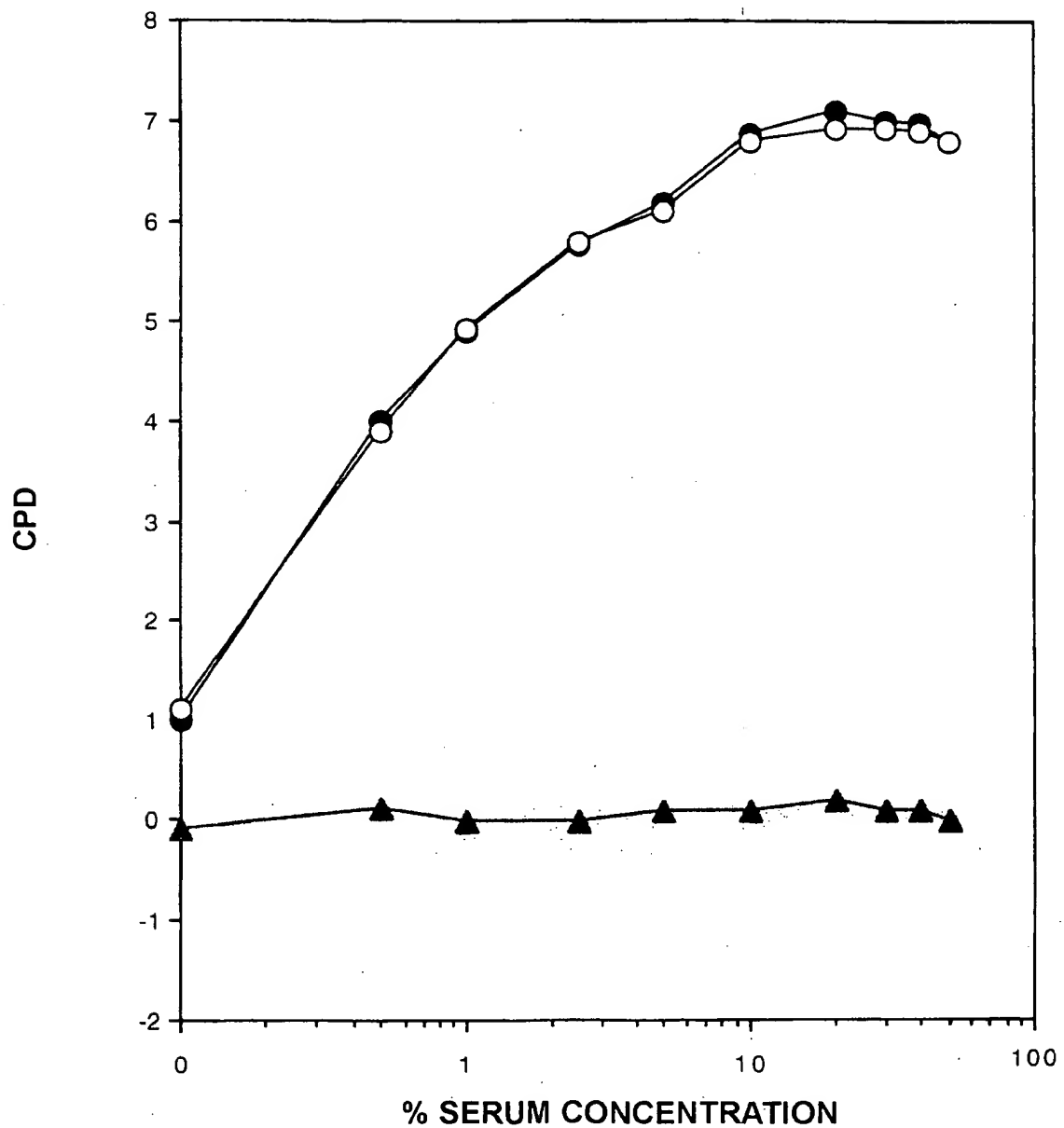
LEGEND:

Open circles = "Low Fe" medium

Closed triangles = "Standard" medium

FIGURE 53

CDE HORSE SERUM TITRATION ON DU145 CELLS

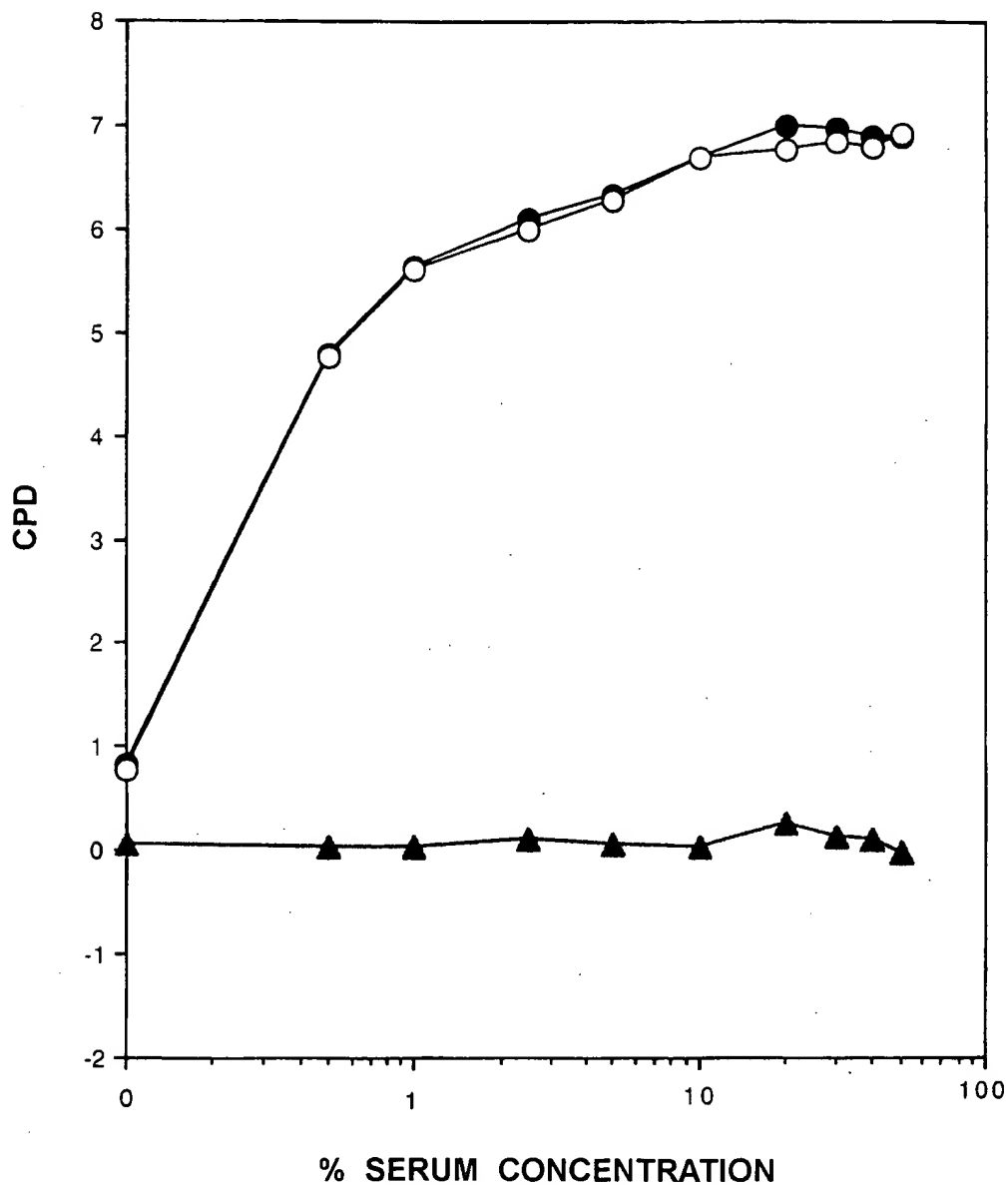


LEGEND:

- = + 10 nM DHT
- = STEROID FREE
- ▲— = ANDROGENIC EFFECT

FIGURE 54

CDE HORSE SERUM TITRATION ON PC3 CELLS

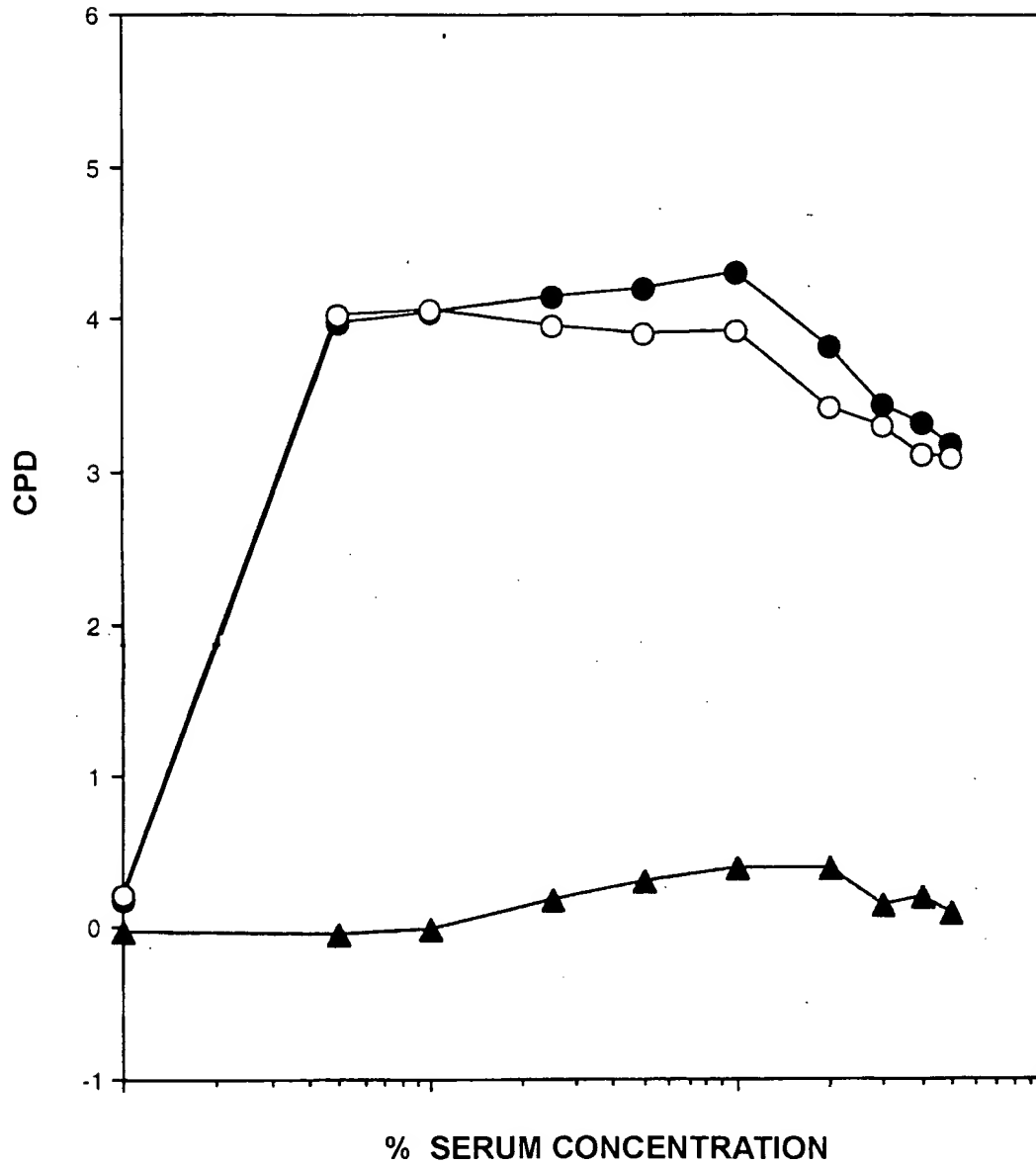


LEGEND:

- = + 10 nM DHT
- = STEROID FREE
- ▲— = ANDROGENIC EFFECT

FIGURE 55

CDE HORSE SERUM TITRATION ON ALVA-41 CELLS

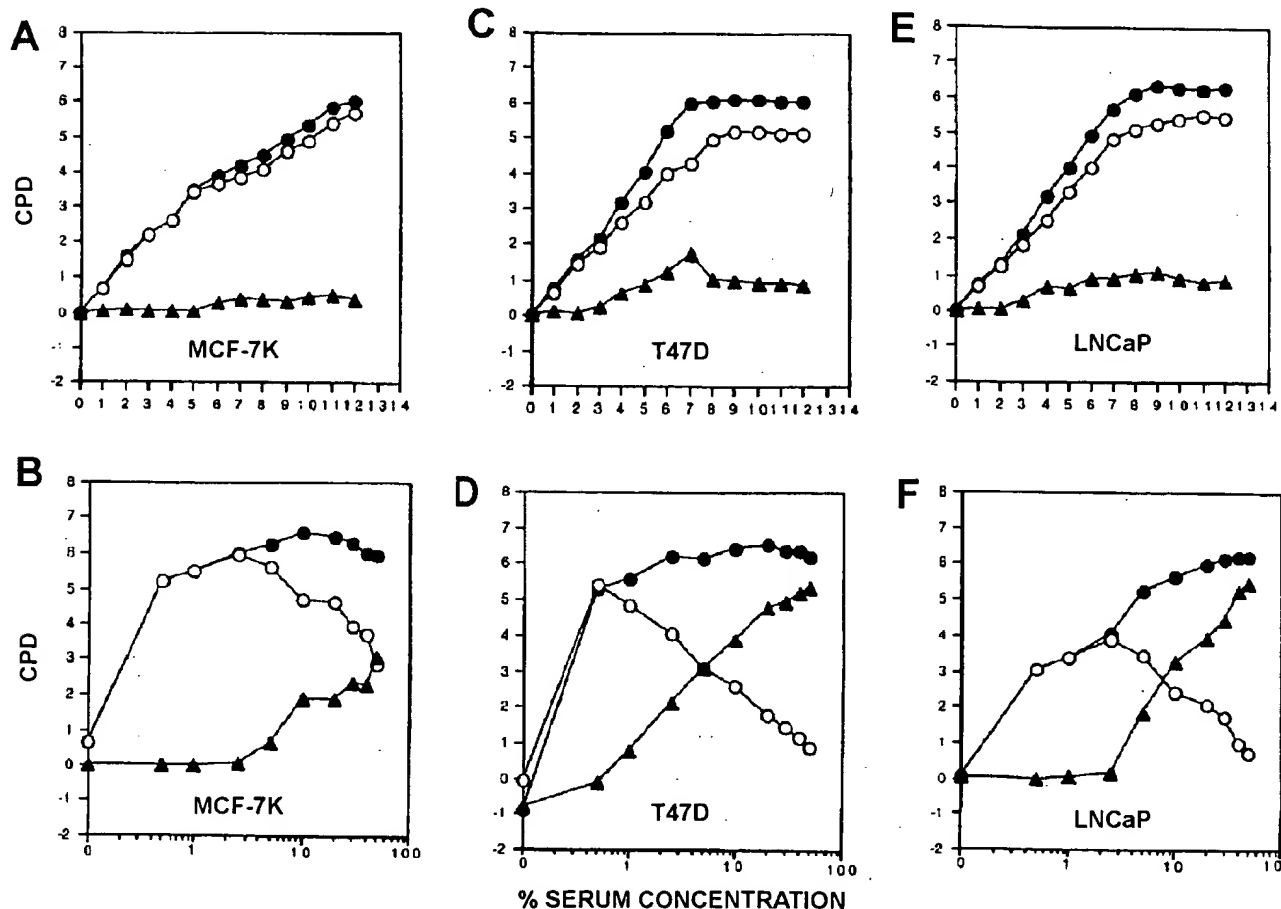


LEGEND:

- = + 10 nM DHT
- = STERIOD FREE
- ▲— = ANDROGENIC EFFECT

FIGURE 56

EFFECTS OF ESTROGEN ON STEROID HORMONE-RESPONSIVE HUMAN TUMOR CELL GROWTH

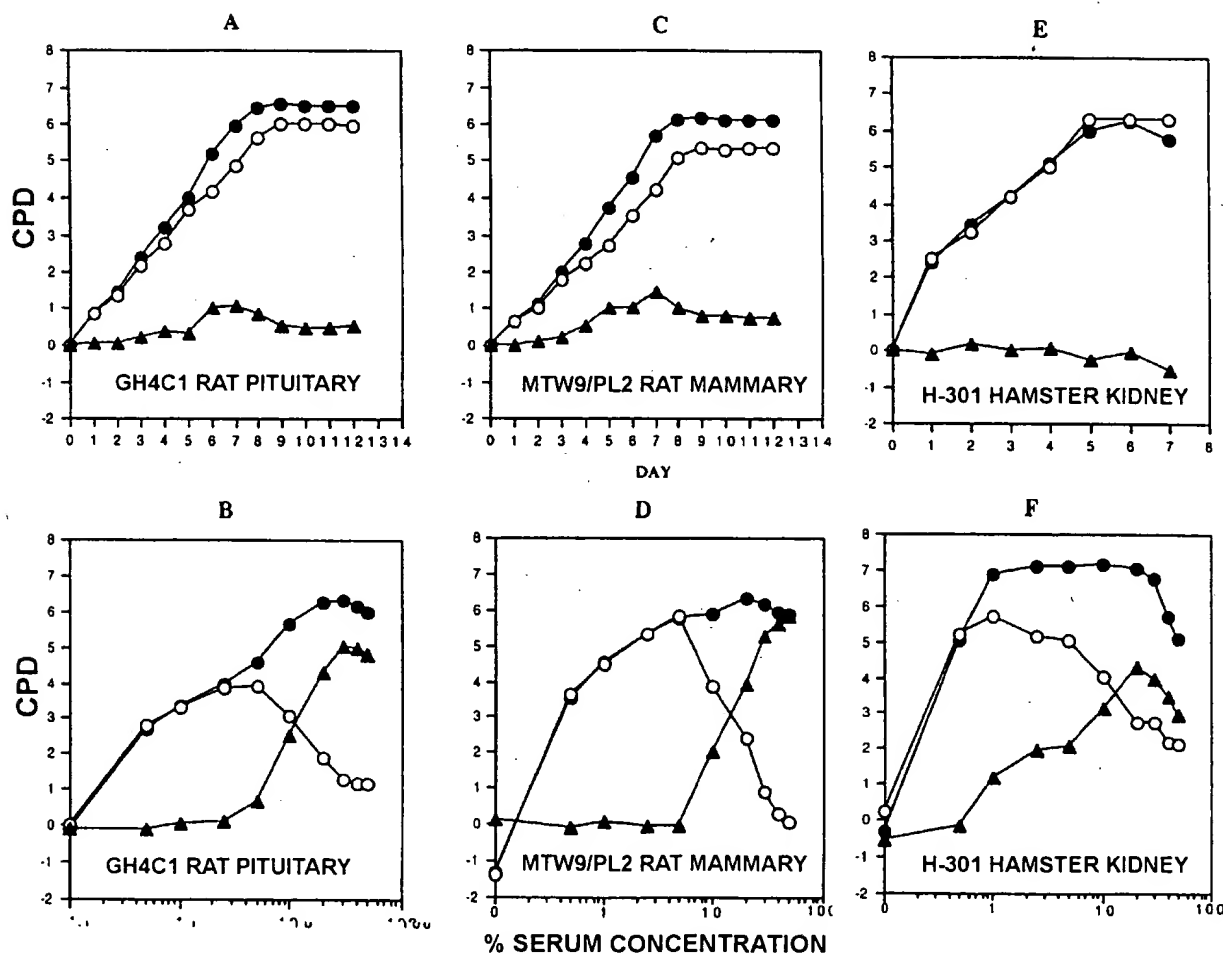


The cells were grown in serum-free defined medium and in D-MEM/F-12 supplemented with increasing concentrations of CDE horse serum.

- (A) MCF-7K cell growth was measured daily in serum-free defined DDM-2MF with 10 nM E₂ (closed circles) and without steroid (open circles) E₂. Triangles = estrogenic effect.
 (B) MCF-7K cell growth measured after 12 d in D-MEM-F-12 supplemented with the designated concentrations of serum with E₂ (closed circles) and without steroid (open circles). The estrogenic effect is shown by triangles.
 (C) and (D) show the same experiments as in (A) and (B), respectively, except with T47D cells.
 (E) and (F) show the same experiments as in (A) and (B), respectively, except with LNCaP cells. In (E) the serum-free medium was CAPM.

FIGURE 57

EFFECTS OF ESTROGEN ON STEROID HORMONE-RESPONSIVE RODENT TUMOR CELL GROWTH

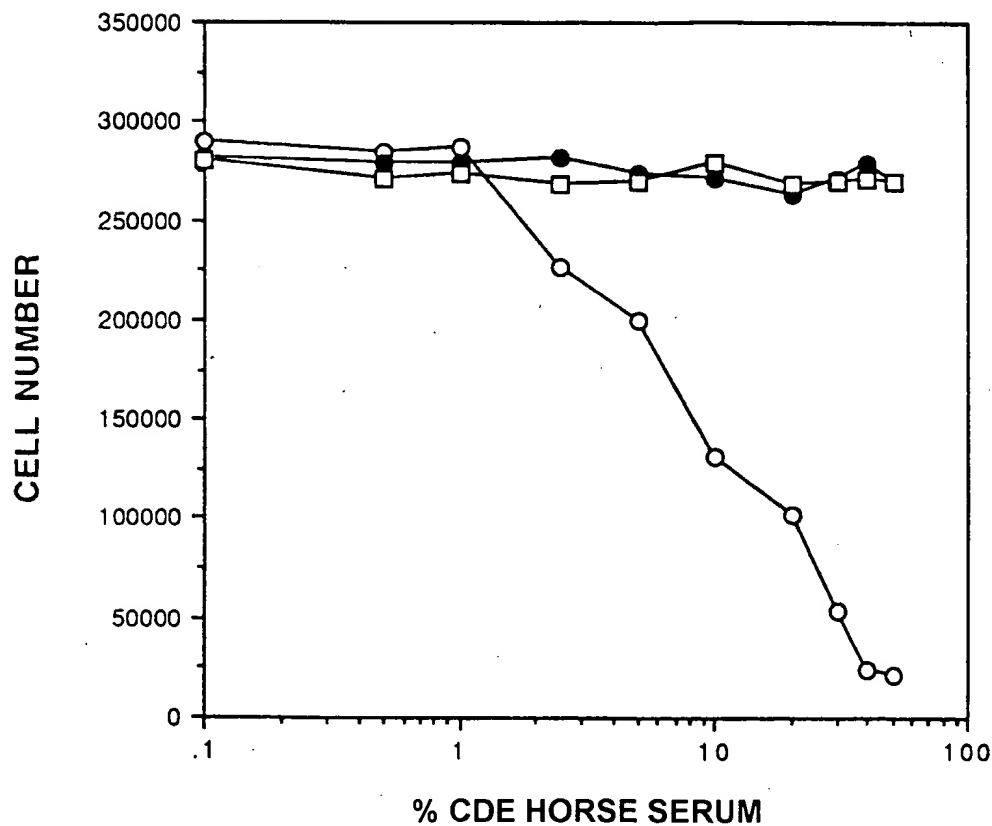


Comparison of the effects of estrogen on steroid hormone-responsive rodent tumor cell growth in serum-free defined medium and in D-MEM/F-12 supplemented with increasing concentrations of CDE horse serum.

- (A) GH₄C₁ rat pituitary tumor cell growth measured daily in serum-free PCM-9 with E_2 (closed circles) and without E_2 (open circles). The estrogenic effect is shown by triangles.
 (B) GH₄C₁ cell growth measured after 9 d in D-MEM-F-12 supplemented with the designated concentrations of CDE horse serum with E_2 (closed circles) and without E_2 (open circles). The estrogenic effect is shown by triangles.
 (C) and (D) show the same experiments as in (A) and (B) respectively, but with the MTW9/PL2 rat mammary tumor cells. The serum-free medium in (D) was DDM-2A.
 (E) and (F) show the same experiments as in (A) and (B), respectively, except with the H-301 hamster kidney tumor cells. In (E) the serum-free medium was CAPM.

FIGURE 58

CDE HORSE SERUM TITRATION ON LNCaP
GROWTH IN SERUM FREE CONDITIONS

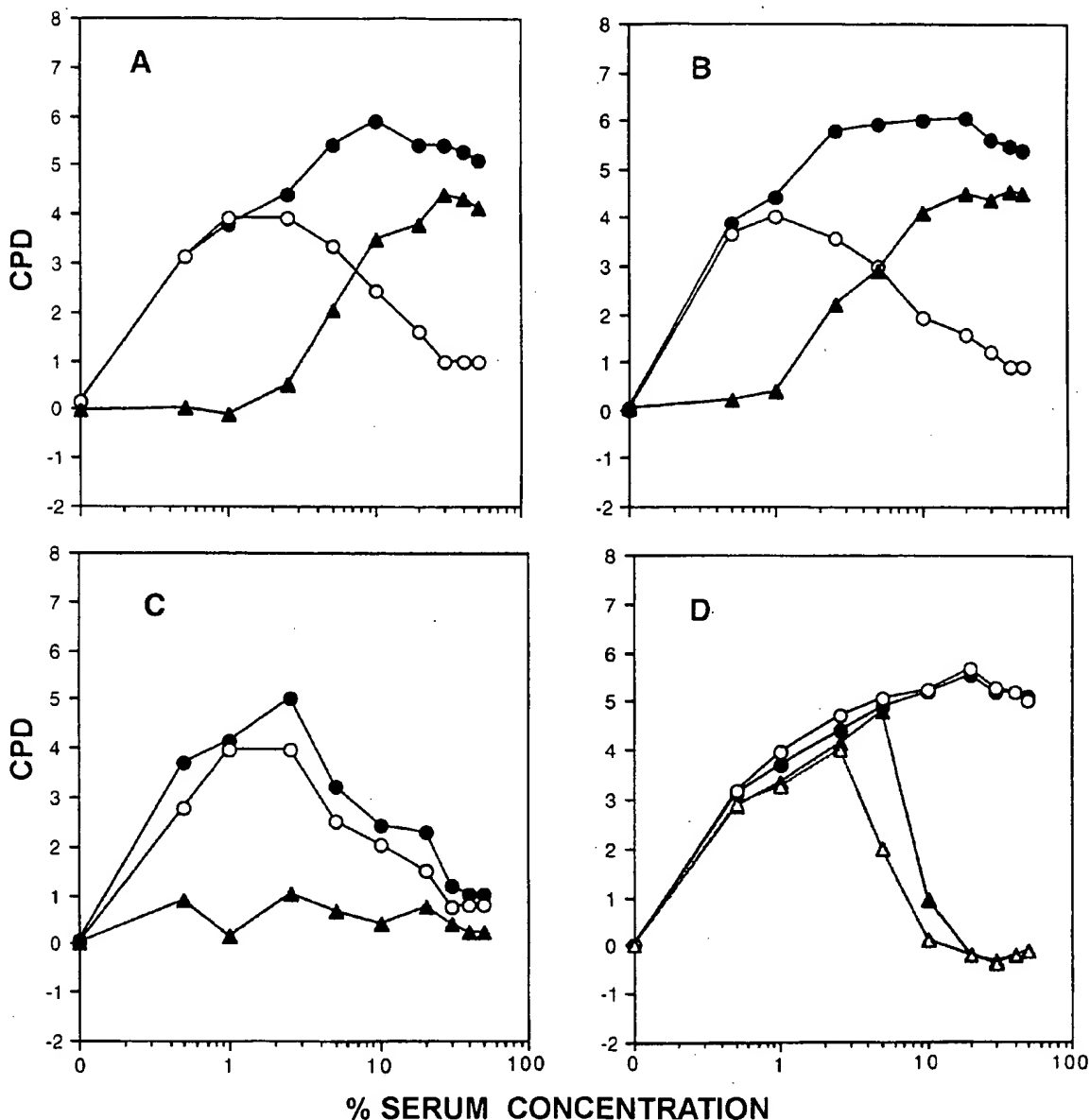


LEGEND:

- NO STEROID
- + E₂
- + DHT

FIGURE 59

**THE EFFECT OF DHT, E₂, AND DES ON
 LNCaP CELLS GROWN IN CDE HORSE SERUM**



LEGEND:

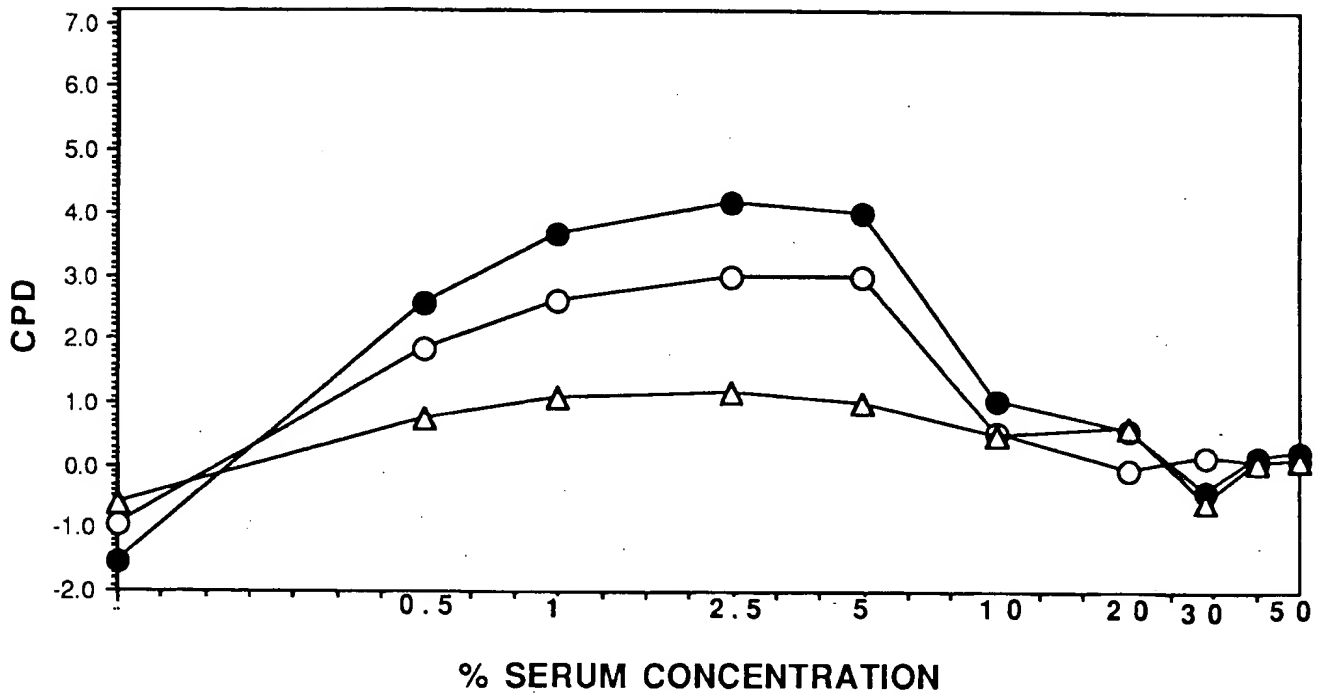
- (A) Open circles = - DHT
 Closed circles = + DHT
 Closed triangles = Androgenic effect

- (B) Open circles = - E₂
 Closed circles = + E₂
 Closed triangles = Estrogenic effect

- (C) Open circles = - DES
 Closed circles = + DES
 Closed triangles = Estrogenic effect
- (D) Open circles = DHT & DES
 Closed circles = E₂ & DES
 Open triangles = No additions
 Closed triangles = DES only

FIGURE 60

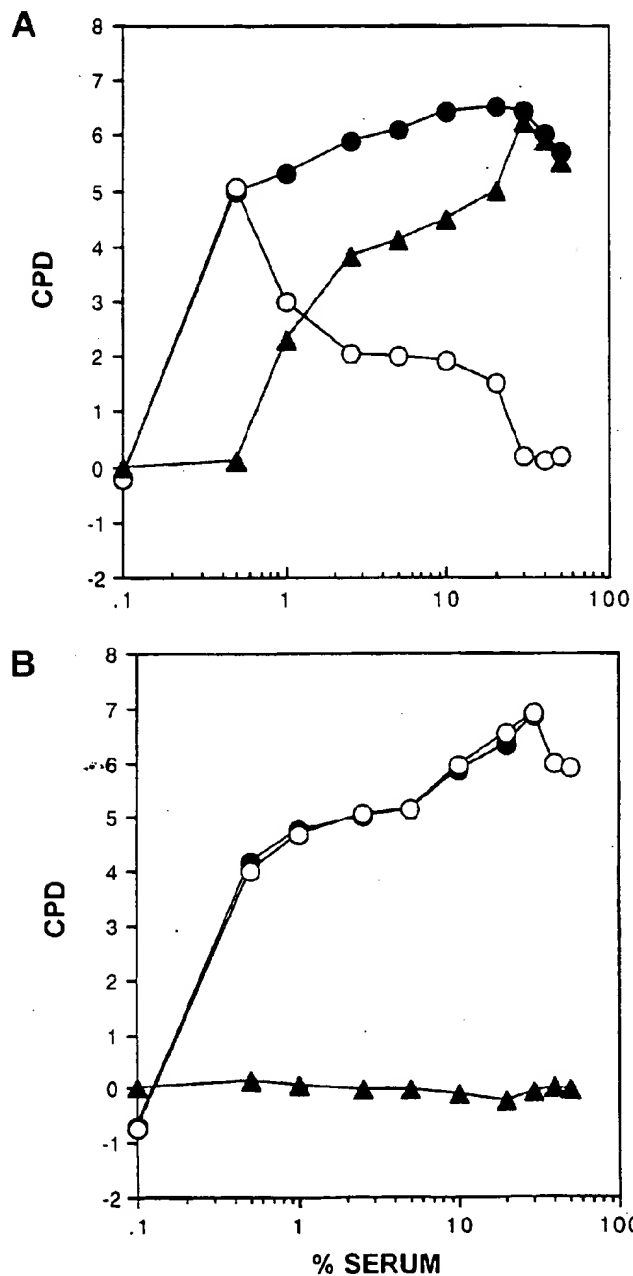
TRIS DIALYSIS OF CDE HORSE SERUM
AND ASSAY WITH MTW9/PL2 CELLS



LEGEND:

- = + E₂
- = - E₂
- △— = Estrogenic effect

FIGURE 61 **ULTRAFILTRATION OF CDE HORSE SERUM AND ESTROGENIC EFFECTS WITH MTW9/PL2 CELLS**



LEGEND:

(A) RETENTATE FROM AMICON MEMBRANE

(B) FILTRATE FROM AMICON MEMBRANE

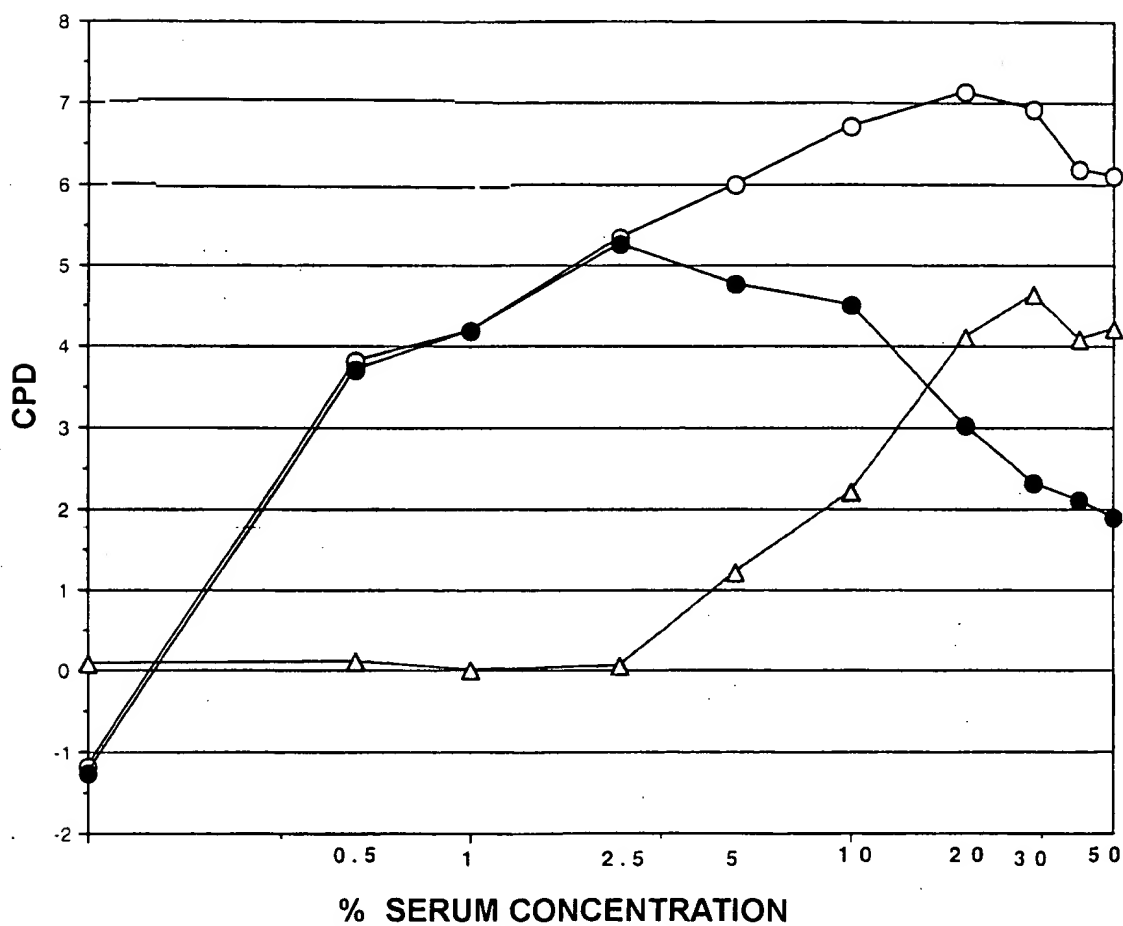
Open circles = - E₂

Closed circles = + E₂

Closed triangles = Estrogenic effect

FIGURE 62

CDE HORSE SERUM TREATED AT 50° C FOR
30 MINUTES AND ASSAYED WITH MTW9/PL2 CELLS

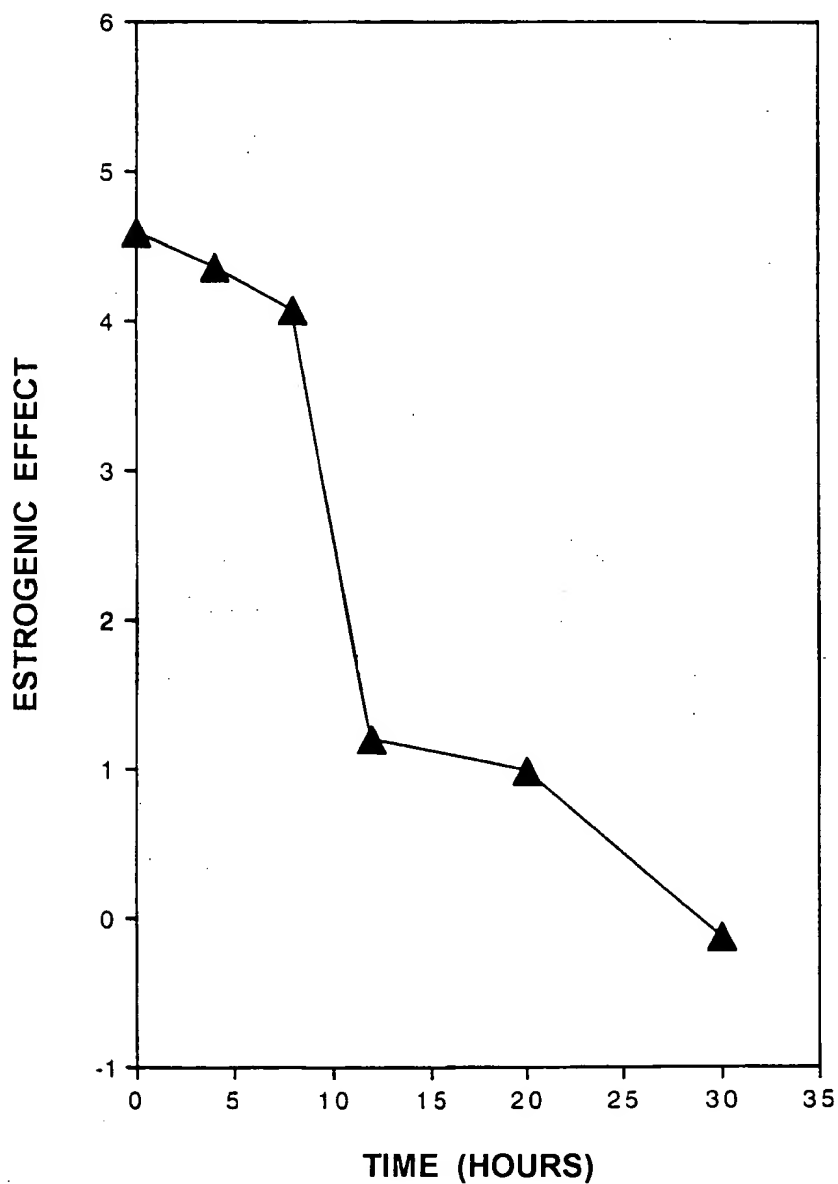


LEGEND:

- = + E₂
- = - E₂
- △— = Estrogenic effect

FIGURE 63

EFFECT OF 50° C INCUBATION ON
ESTROGENIC EFFECT WITH MTW9/PL2

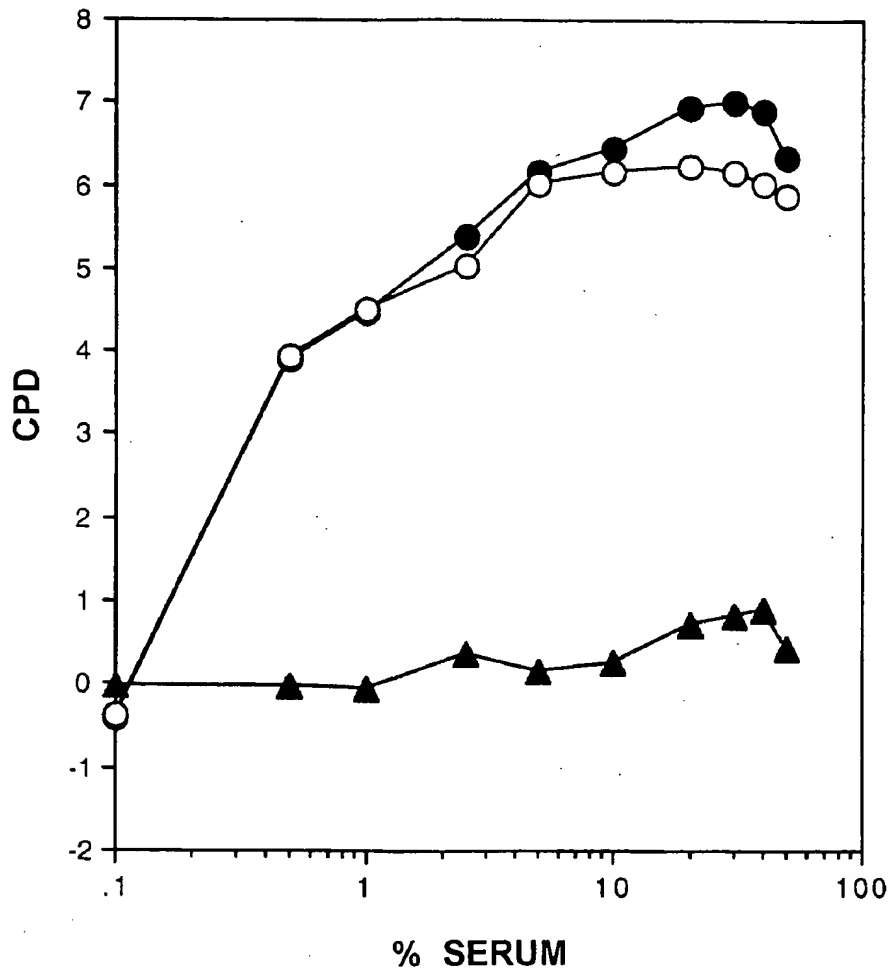


LEGEND:

Closed triangles = Estrogenic effect

FIGURE 64

CDE HORSE SERUM INCUBATION AT 50° C
FOR 20 HOURS AND ASSAYED WITH MTW9/PL2



LEGEND:

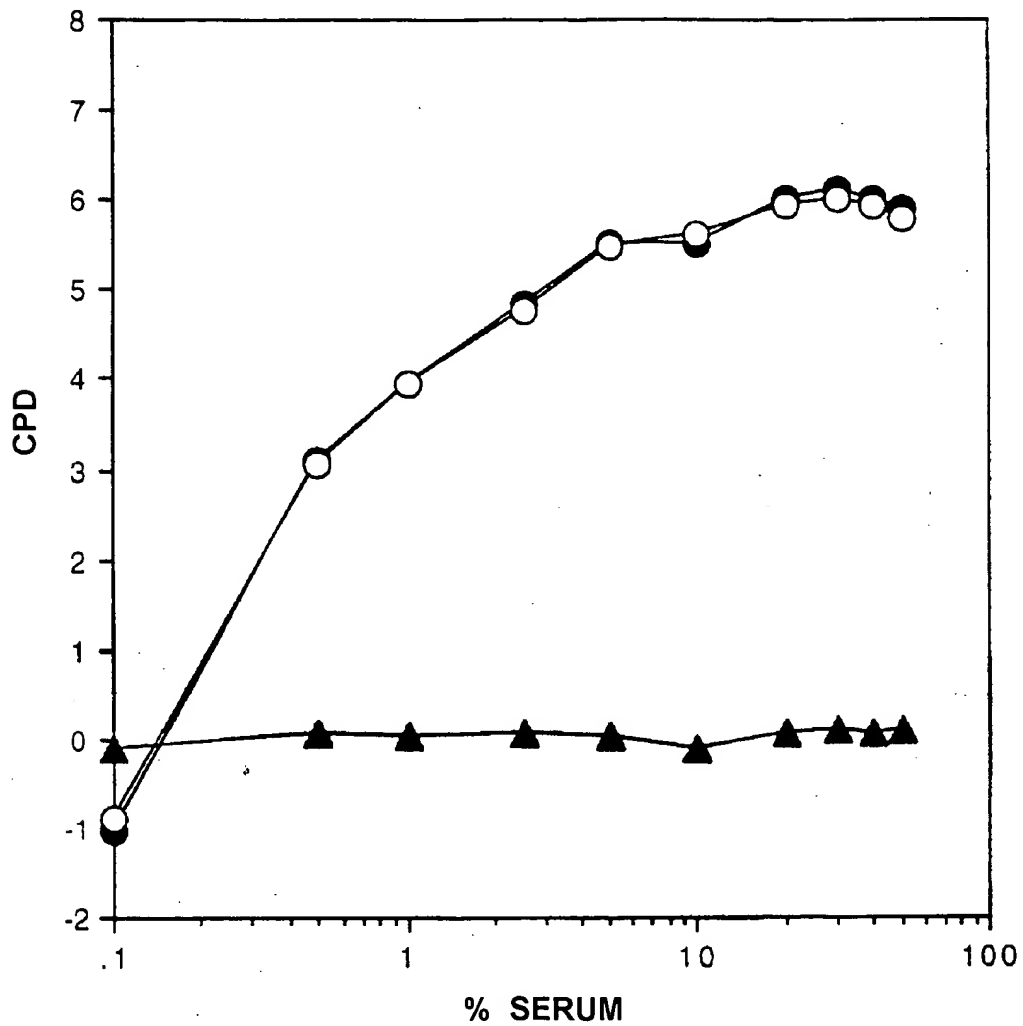
Open circles = - E₂

Closed circles = + E₂

Closed triangles = Estrogenic effect

FIGURE 65

CDE HORSE SERUM INCUBATED AT 60° C FOR
90 MINUTES AND ASSAYED WITH MTW9/PL2 CELLS



LEGEND:

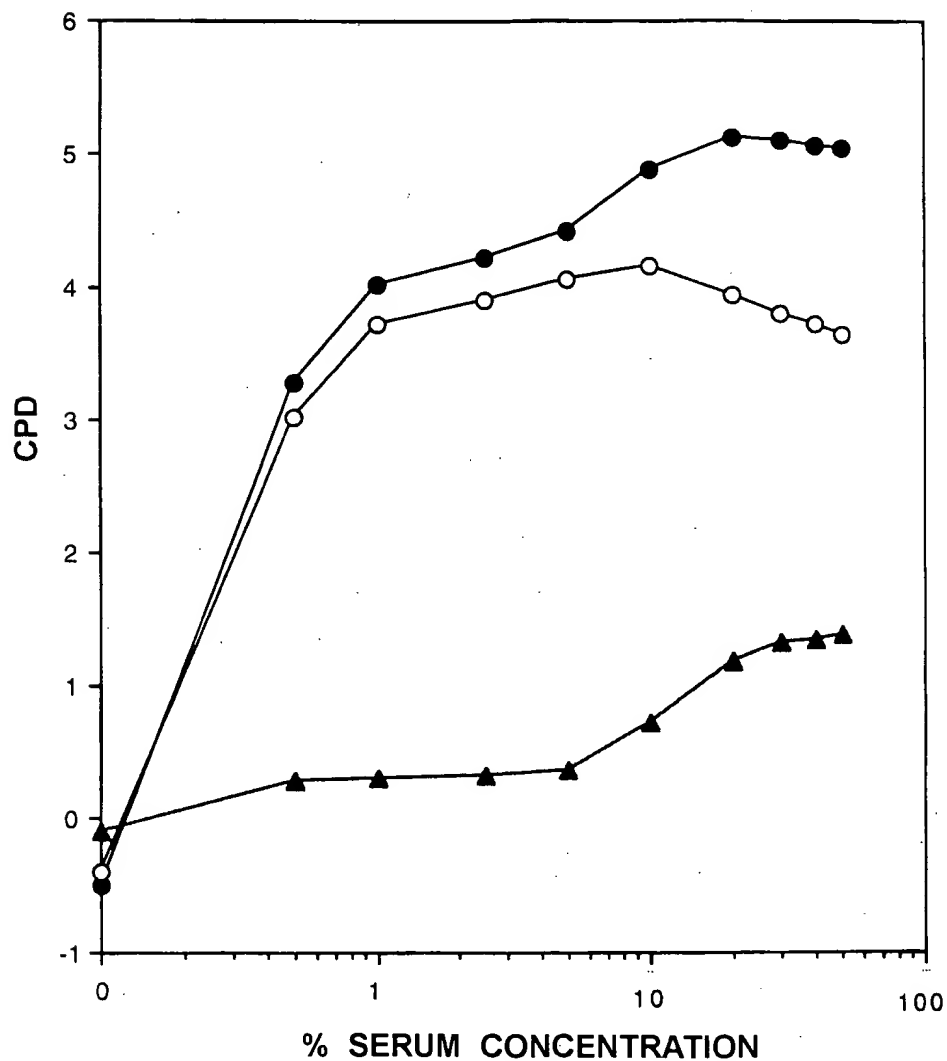
Open circles = - E₂

Closed circles = + E₂

Closed triangles = Estrogenic effect

FIGURE 66

AFFI-GEL BLUE TREATMENT OF CDE HORSE
SERUM AND ASSAY WITH MTW9/PL2 CELLS



LEGEND:

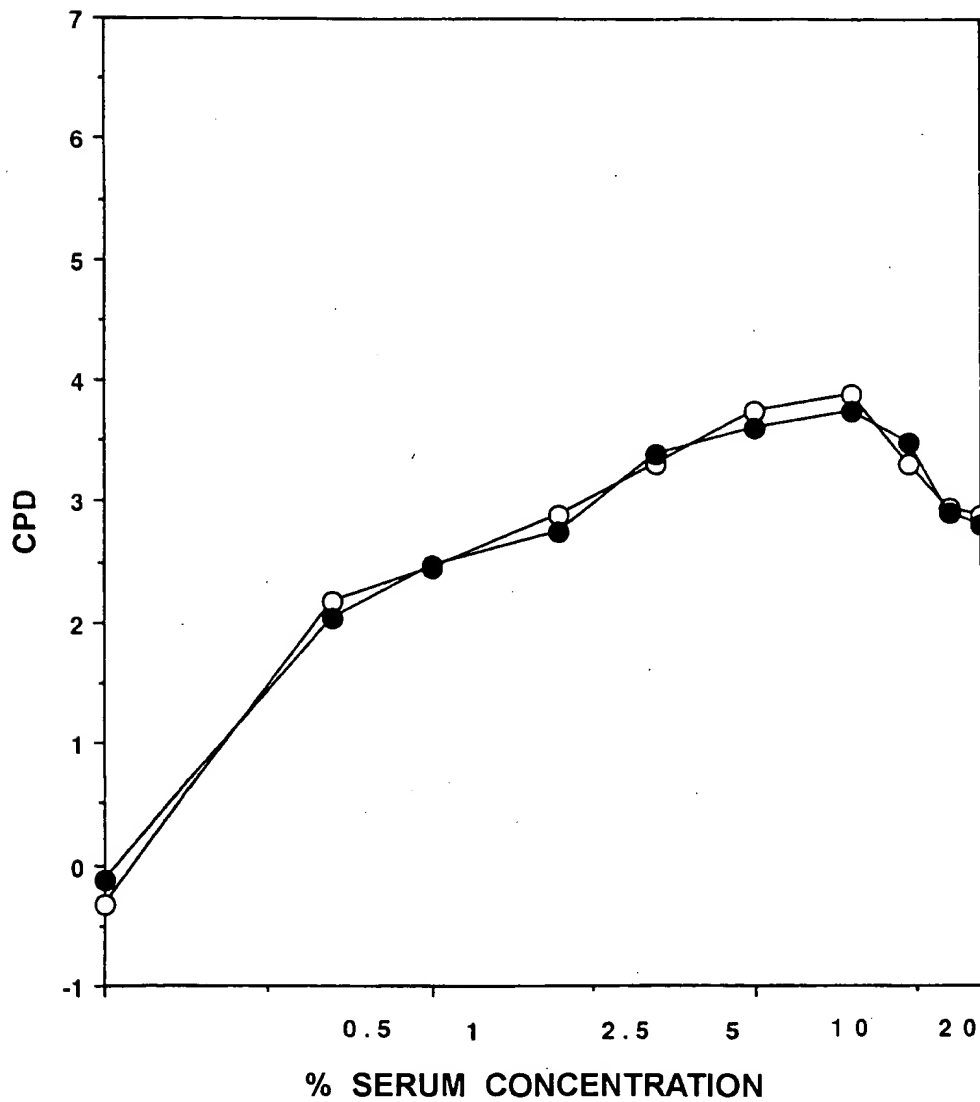
Open circles = - E₂

Closed circles = + E₂

Closed triangles = Estrogenic effect

FIGURE 67

DIALYSIS OF CDE HORSE SERUM AGAINST
6M UREA AND ASSAY WITH MTW9/PL2 CELLS

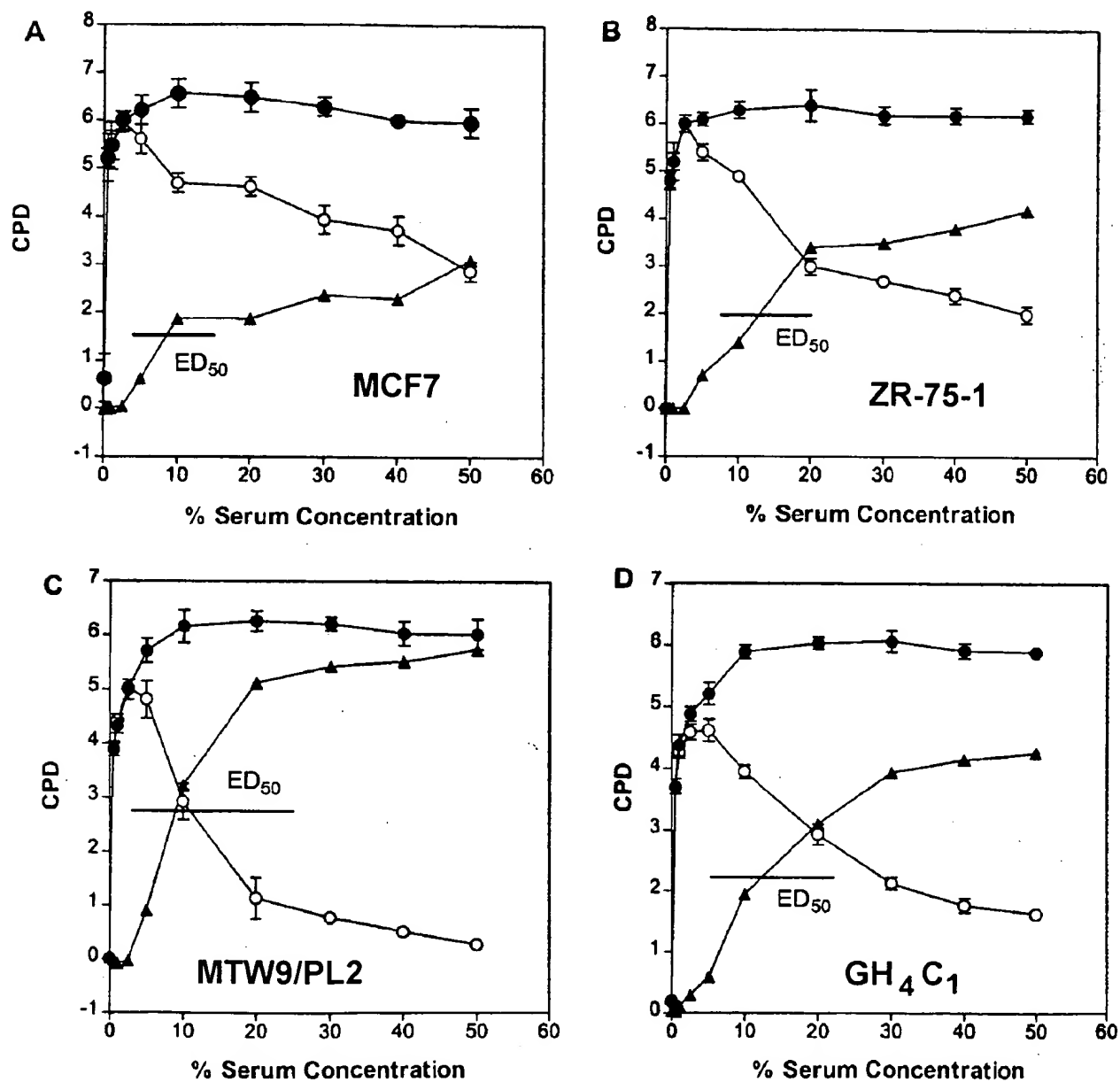


LEGEND:

—○— = + E₂
—●— = - E₂

FIGURE 68

ED₅₀ MEASUREMENTS OF THE ESTROGENIC EFFECTS
 OF CDE HORSE SERUM WITH VARIOUS CELL LINES



LEGEND:

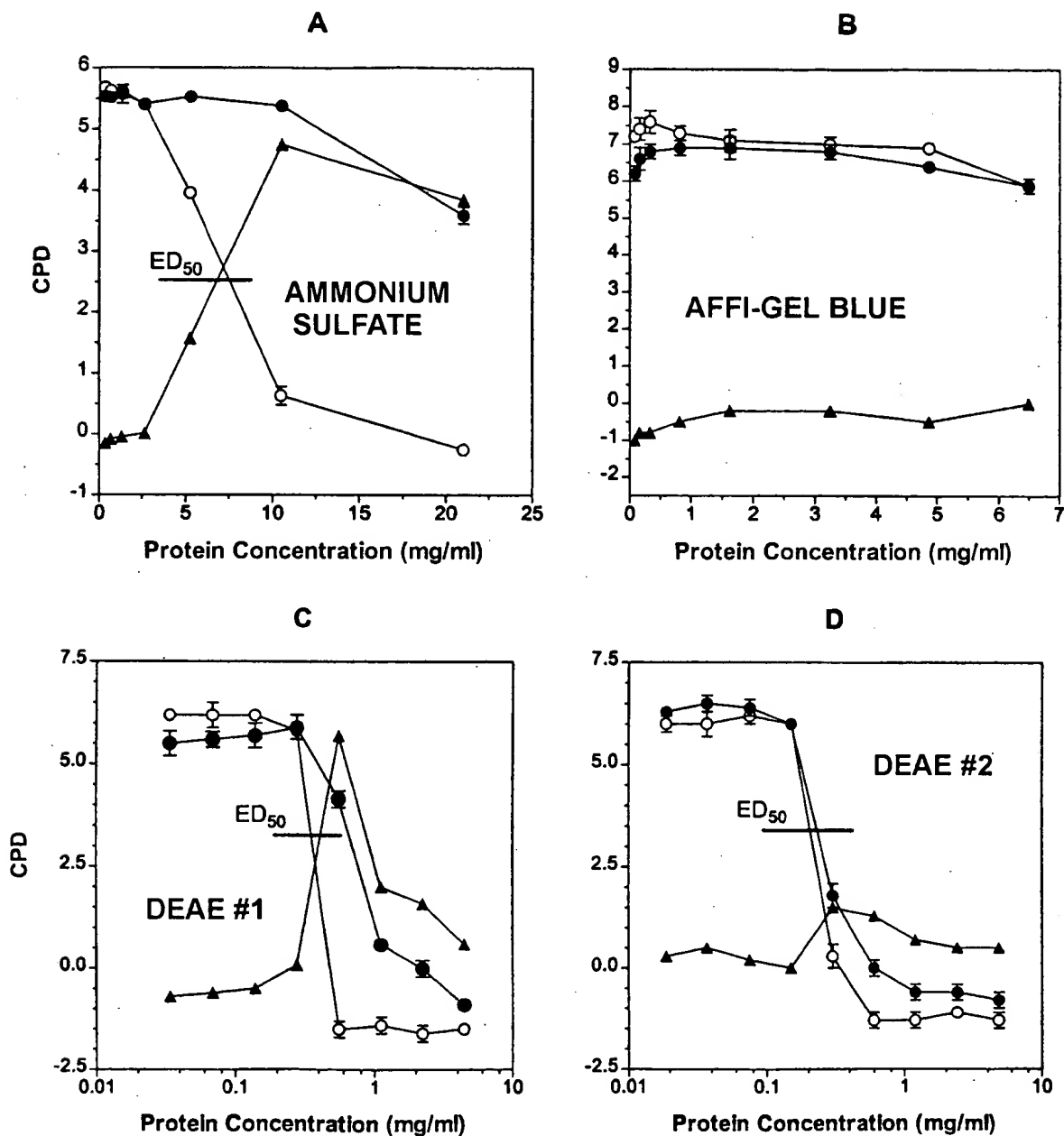
Closed circles = + E₂

Open circles = - E₂

Closed triangles = Estrogenic effect

FIGURE 69

ASSAY OF ESTROGENIC ACTIVITY
 (ED₅₀) OF CHROMATOGRAPHIC POOLS



LEGEND:

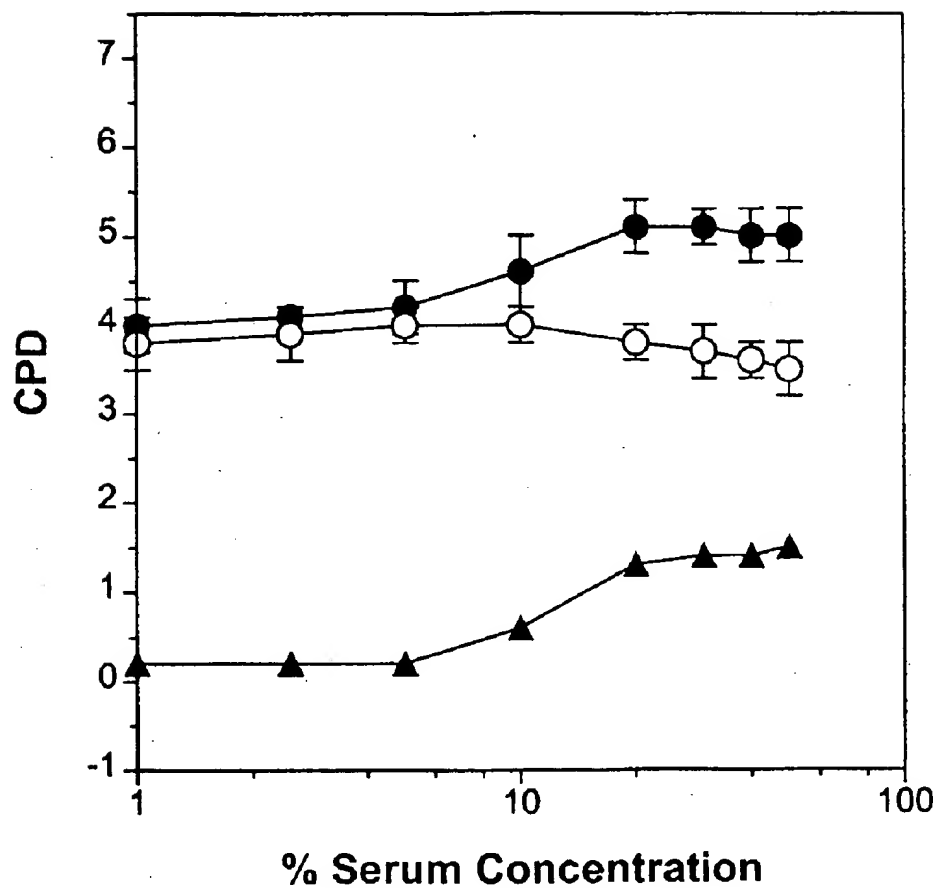
Closed circles = + E₂

Open circles = - E₂

Closed triangles = Estrogenic effect

FIGURE 70

AFFI-GEL BLUE BYPASS FRACTION
ASSAYED WITH MTW9/PL2 CELLS



LEGEND:

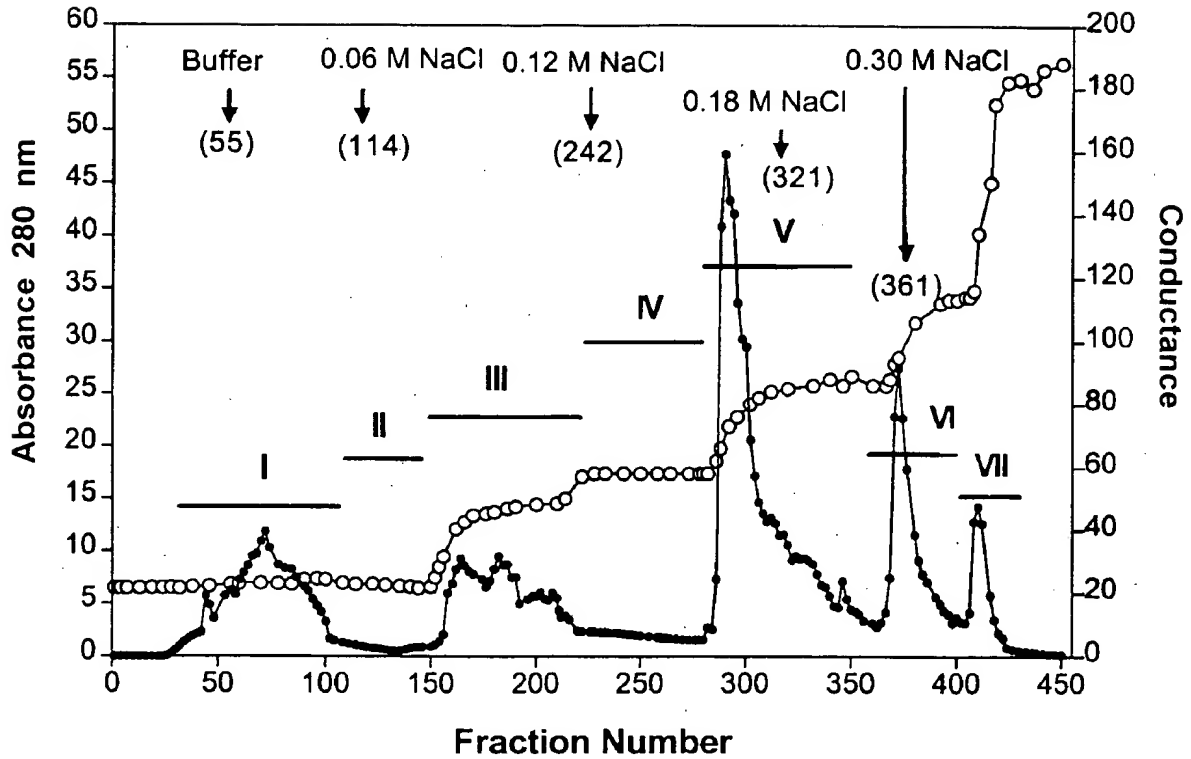
Closed circles = + E₂

Open circles = - E₂

Closed triangles = Estrogenic effect

FIGURE 71

**DEAE SEPHAROSE CHROMATOGRAPHY
OF CDE HORSE SERUM**



LEGEND:

BARS = FRACTION POOLS

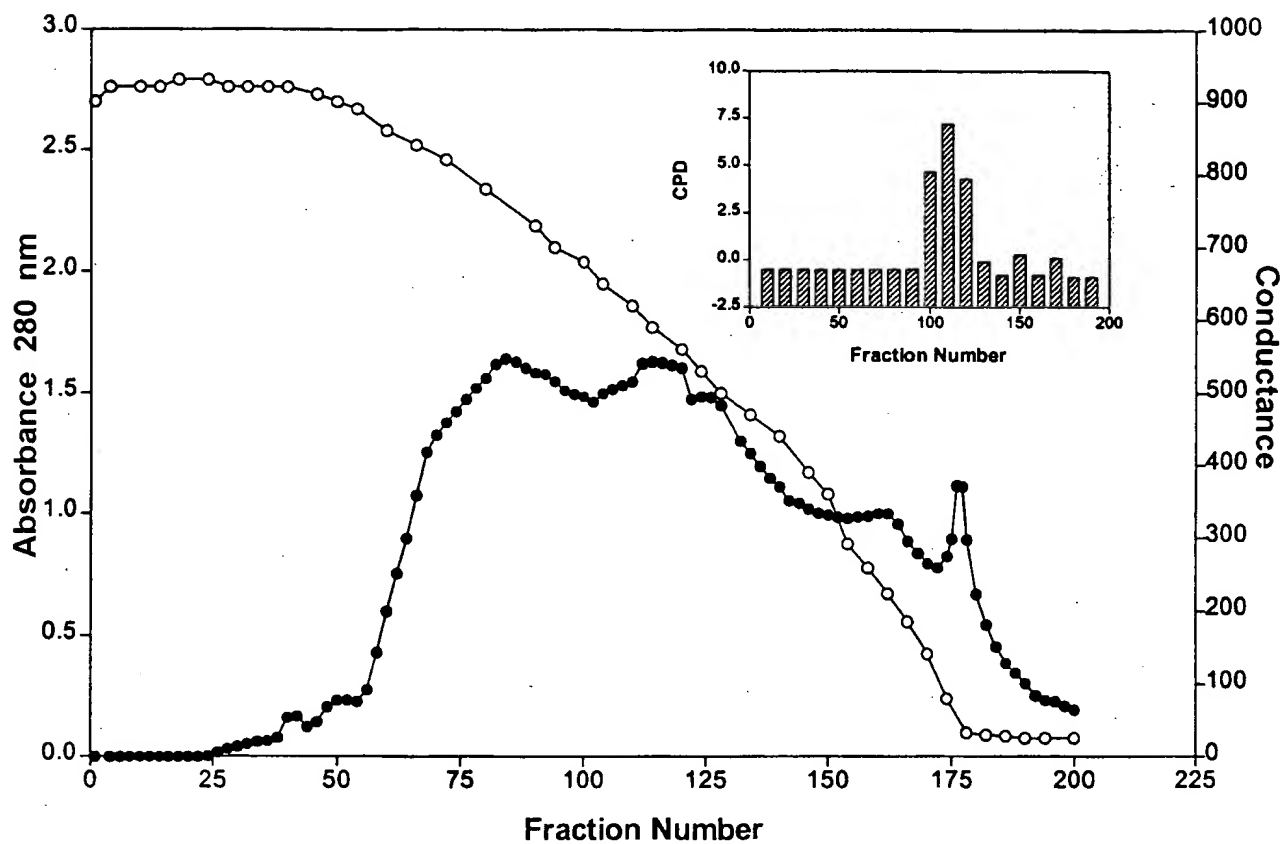
ARROWS = BUFFER CHANGES

Closed circles = Absorbance at 280 nm

Open circles = Conductance

FIGURE 72

THE ELUTION PROFILE OF PHENYL
SEPHAROSE WITH THE DEAE IV POOL



INSERT: Estrogenic activity with MTW9/PL2

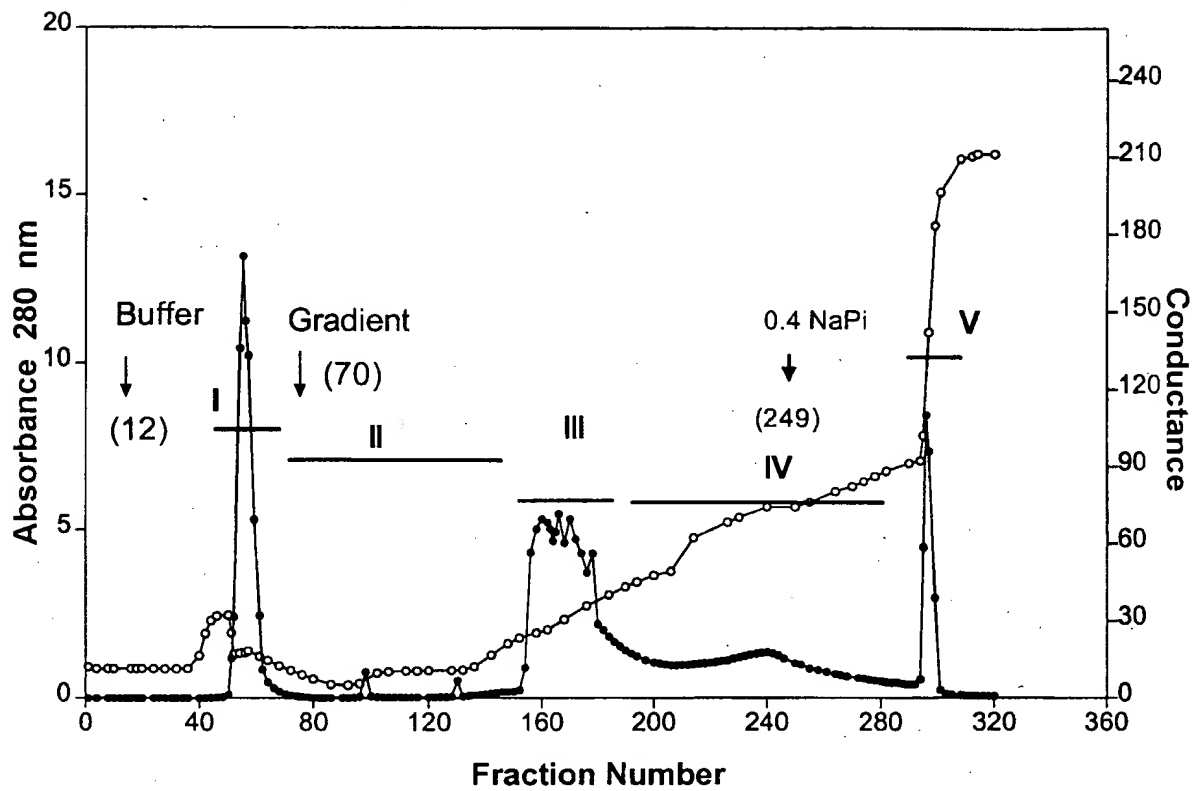
LEGEND:

Closed circles = Absorbance 280 nm

Open circles = Conductance

FIGURE 73

HTP BIO-GEL CHROMATOGRAPHY OF DEAE POOL IV



BARS = FRACTION POOLS

ARROWS = BUFFER CHANGES

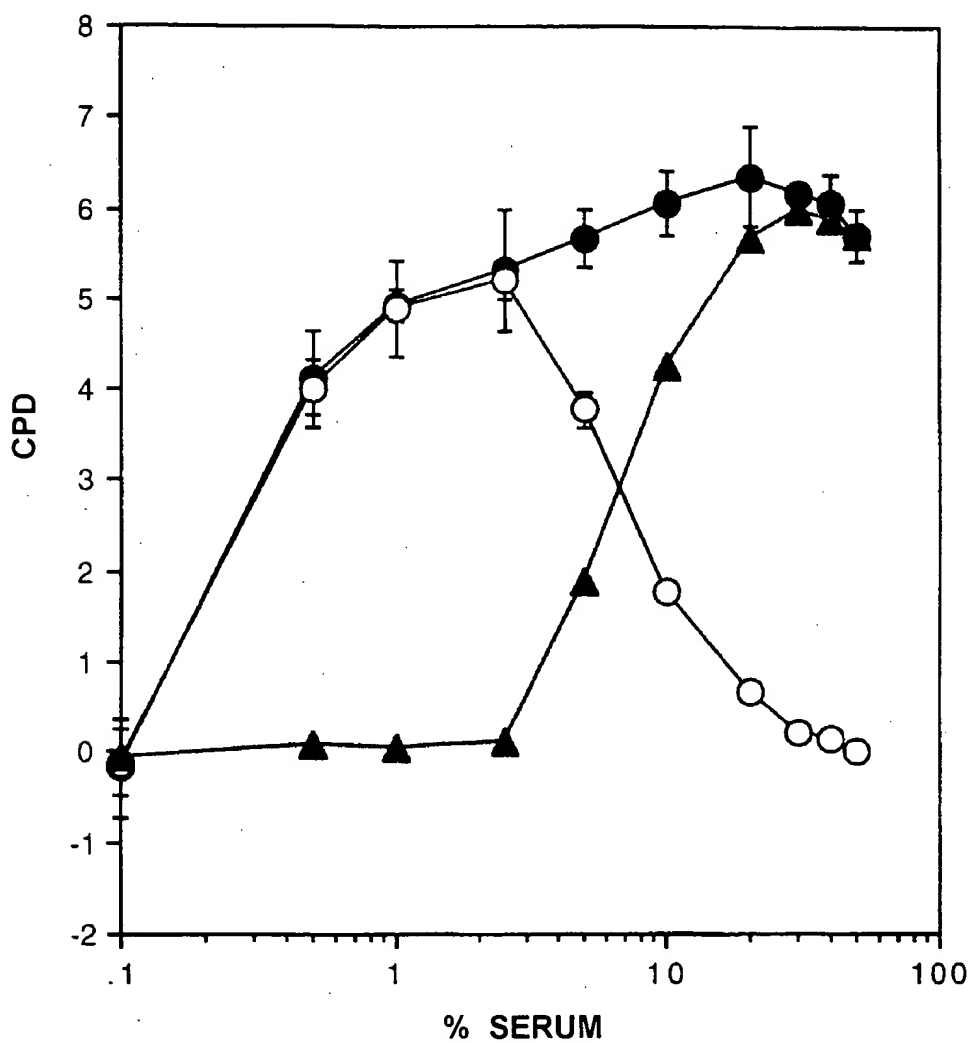
LEGEND:

Open circles = Conductance

Closed circles = Absorbance

FIGURE 74

DIALYSIS OF CDE HORSE SERUM
AGAINST TRIS BUFFER CONTAINING CALCIUM



LEGEND:

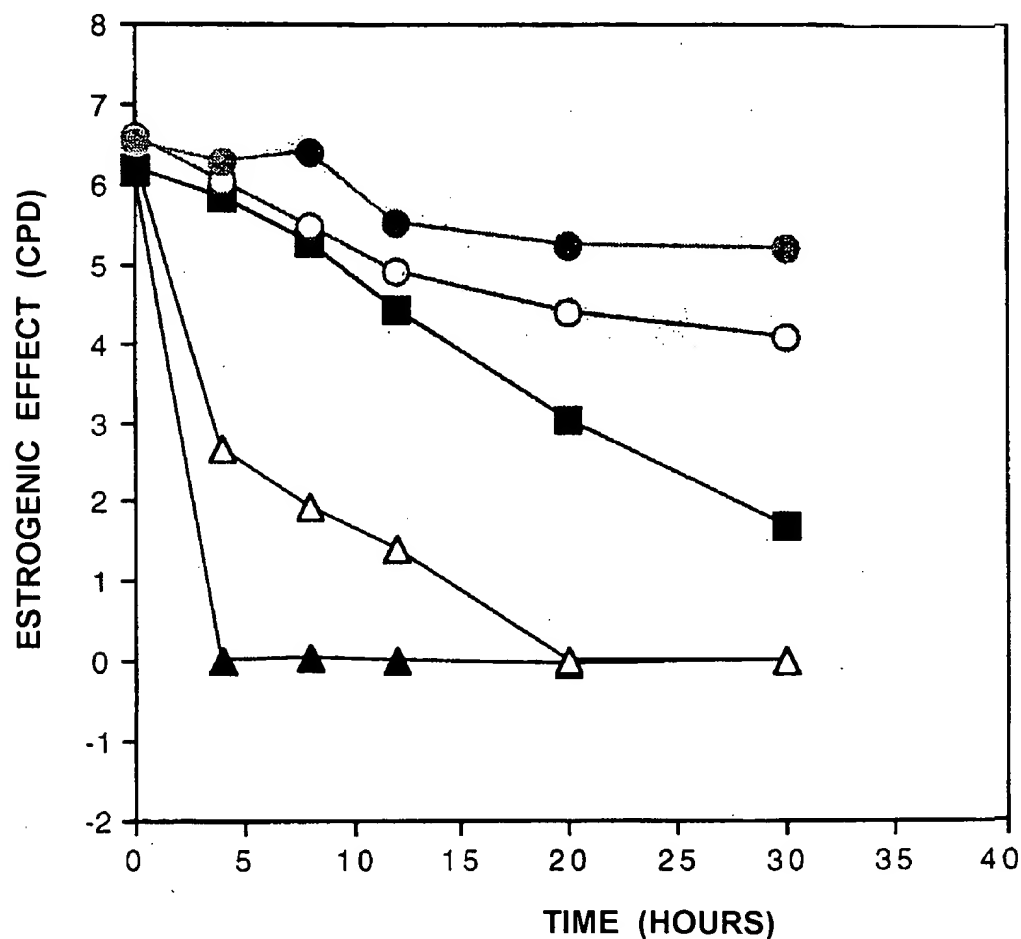
Open circles = - E₂

Closed circles = + E₂

Closed triangles = Estrogenic effect

FIGURE 75

THE EFFECT OF CALCIUM ON THE HEAT STABILITY OF
THE INHIBITOR IN CDE HORSE SERUM (MTW9/PL2 CELLS)

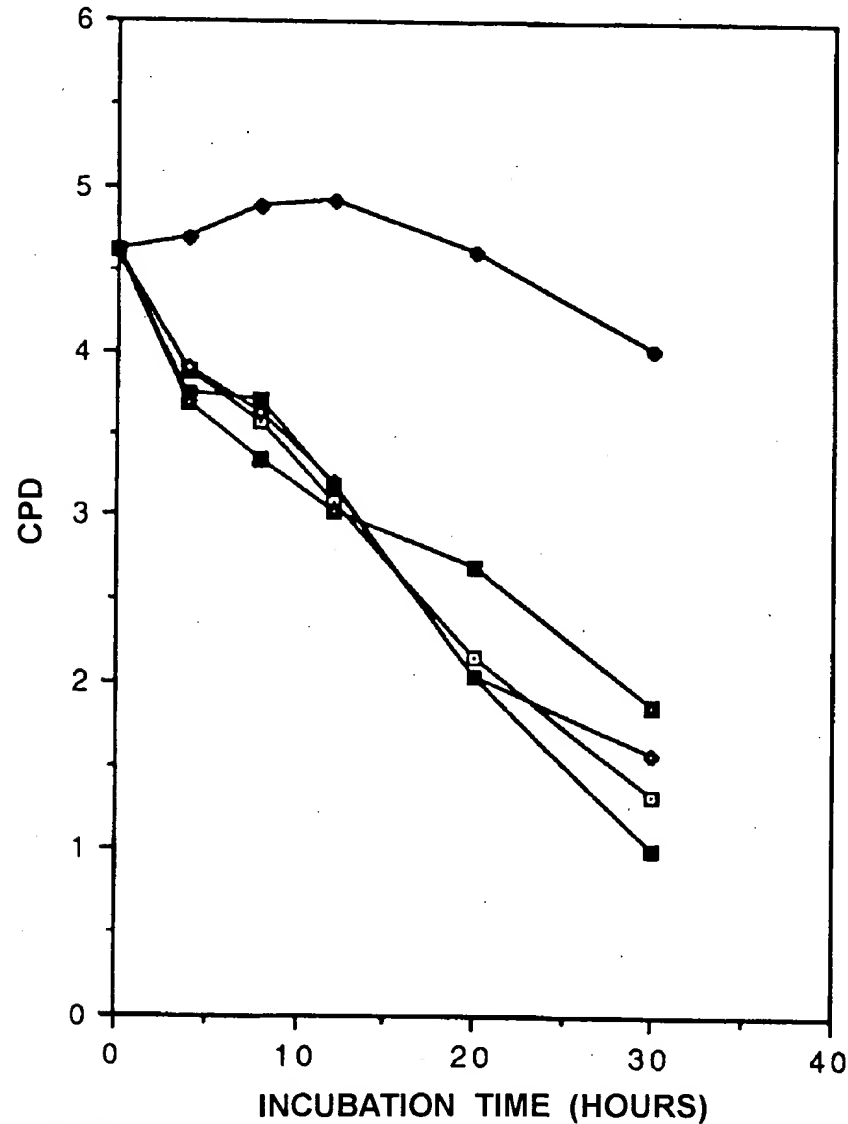


LEGEND:

- ▲— = Chelex treatment only
- △— = CDE horse serum
- = Chelex and 1 mM calcium chloride
- = Chelex and 10 mM calcium chloride
- = Chelex and 50 mM calcium chloride

FIGURE 76

PROTECTIVE EFFECT OF METAL IONS ON CHELEX TREATED CDE HORSE SERUM INCUBATED AT 37° C AND ASSAYED WITH MTW9/PL2 CELLS

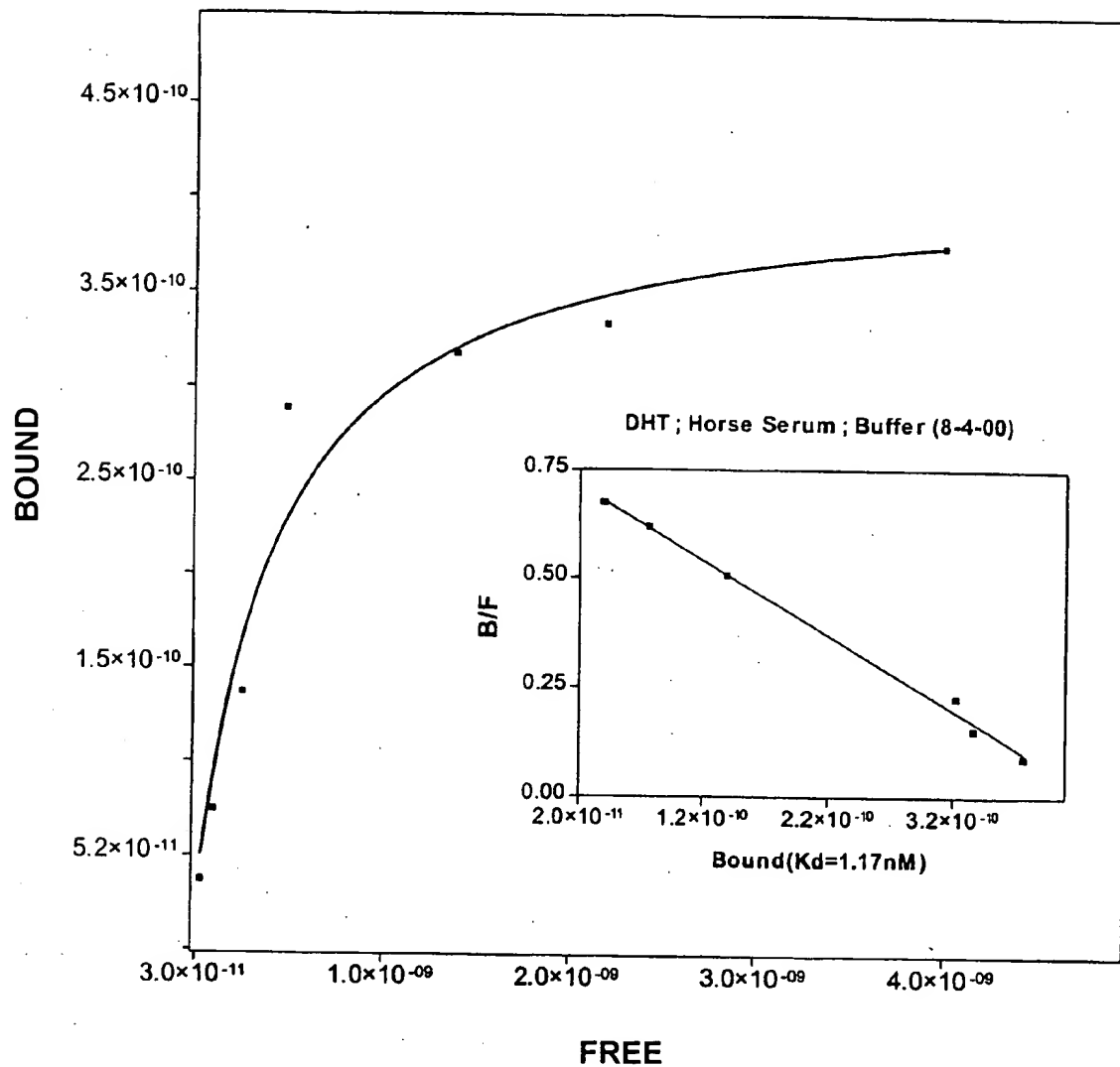


LEGEND:

- Chelex treated serum
- Chelex treated serum + 10 mM Calcium
- Chelex treated serum + 50 uM Manganese
- Chelex treated serum + 100 uM Magnesium
- Chelex treated serum + 10 uM Zinc

FIGURE 77

LABELED DHT BINDING TO CDE HORSE SERUM
SATURATION ANALYSIS AND SCATCHARD PLOT



INSERT:

Scatchard analysis of DHT binding

FIGURE 78

EFFECT OF CALCIUM ON ESTROGENIC EFFECT (A)
AND LABELED STEROID HORMONE BINDING (B)

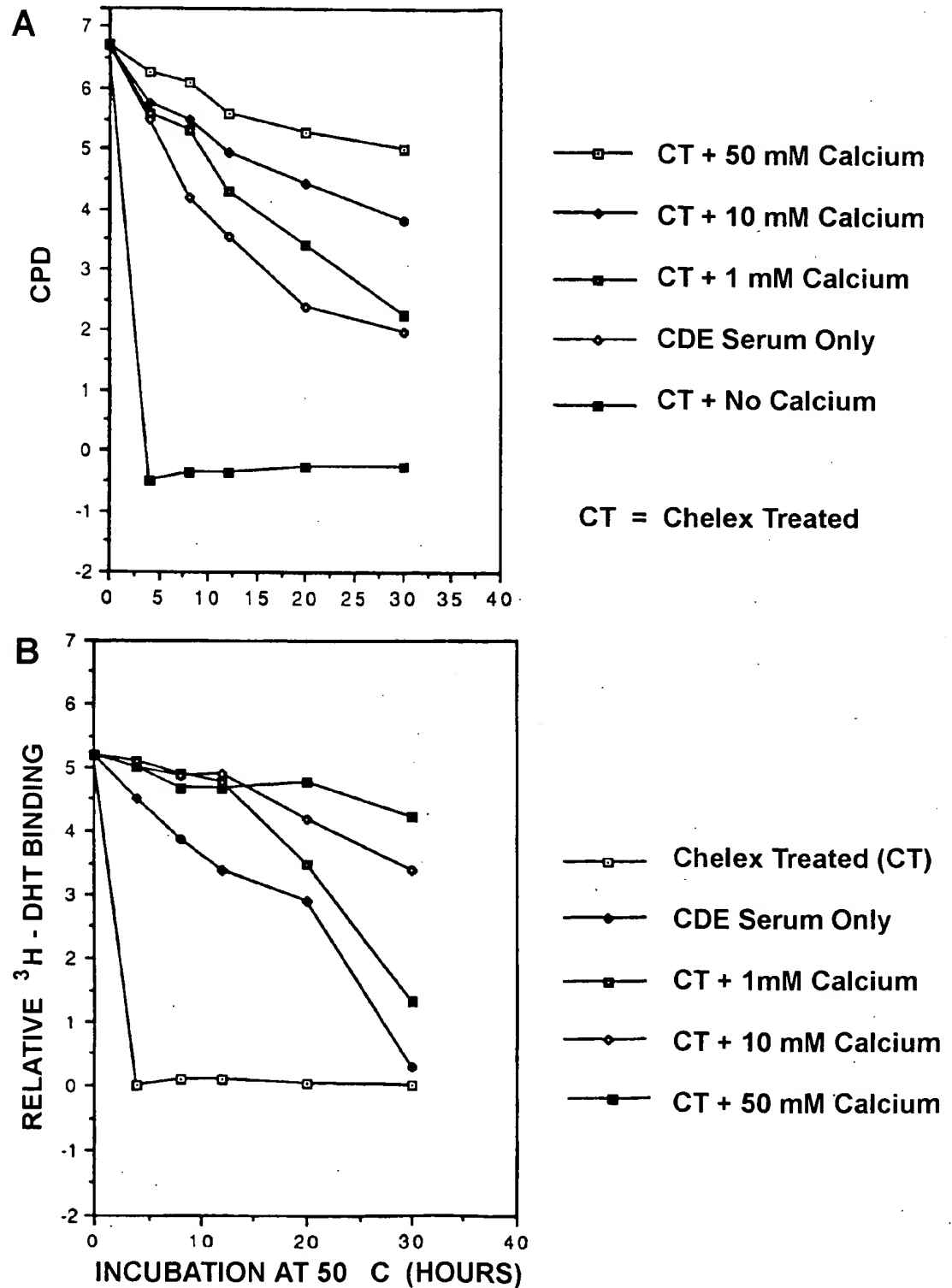


FIGURE 79

**ANTI - HUMAN SHBG PRECIPITATION OF THE
LABELED DHT BINDING ACTIVITY (A) AND THE
ESTROGENIC EFFECT IN CDE HORSE SERUM (B)**

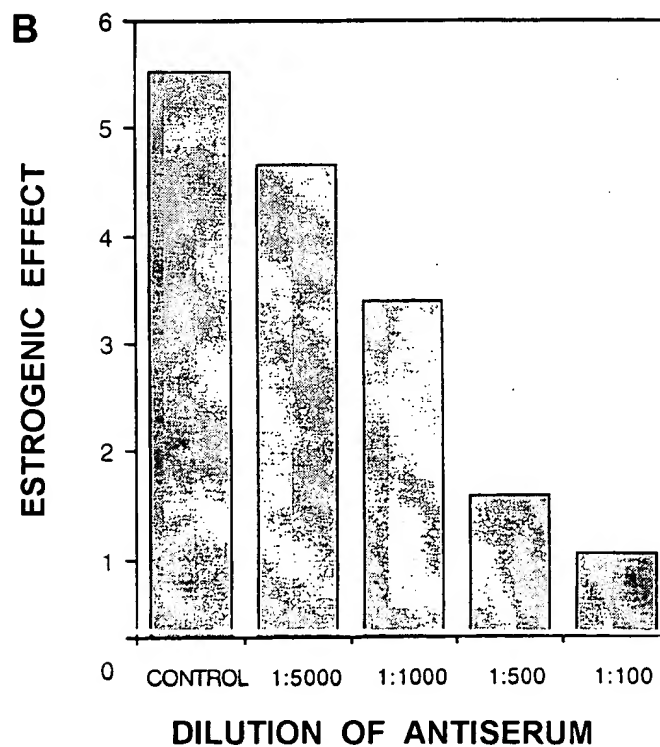
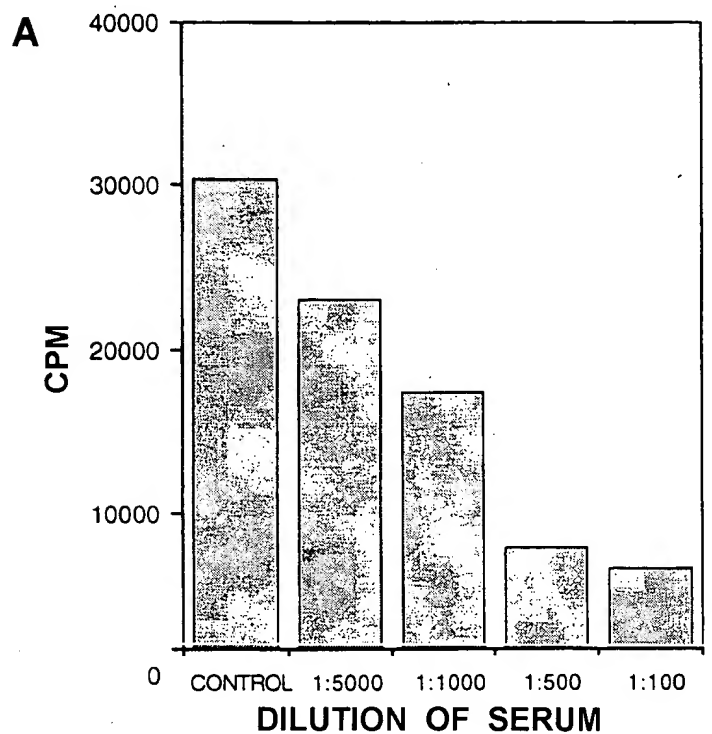
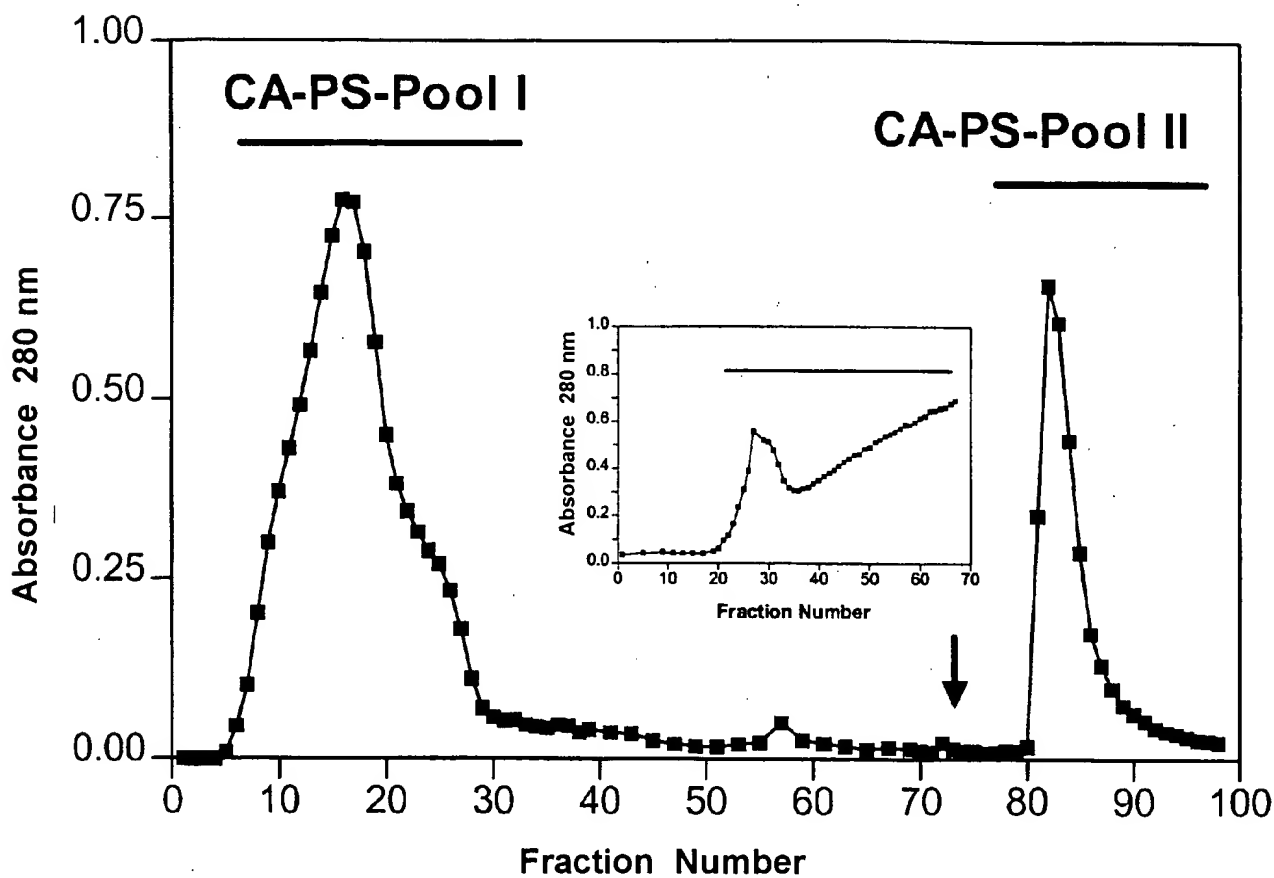


FIGURE 80

**PHENYL SEPHAROSE ELUTION OF
CBG (CA-PS-POOL 1) AND SHBG-LIKE (CA-PS-POOL 11)**



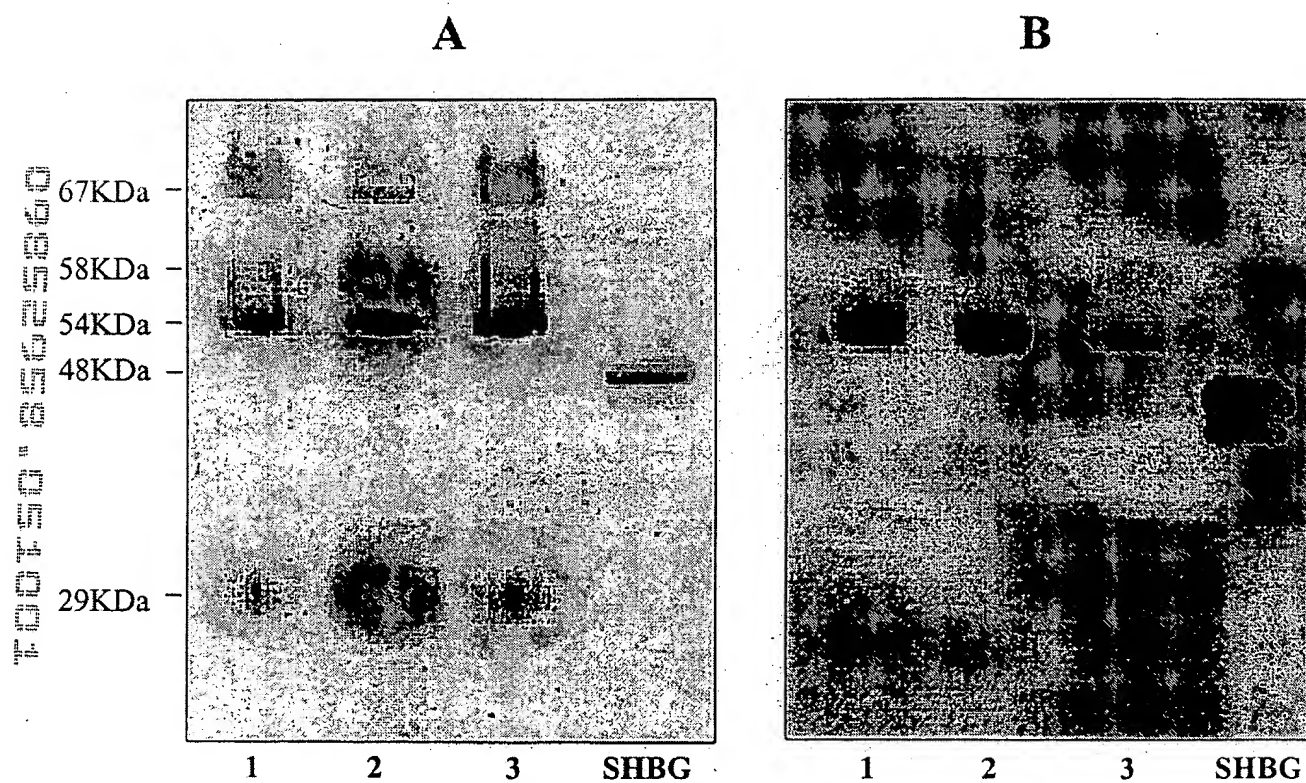
ARROW = ELUTION WITH 40% ETHYLENE GLYCOL

INSERT: CORTISOL AFFINITY COLUMN ELUTION

BARS = POOLED ACTIVE FRACTION

FIGURE 81

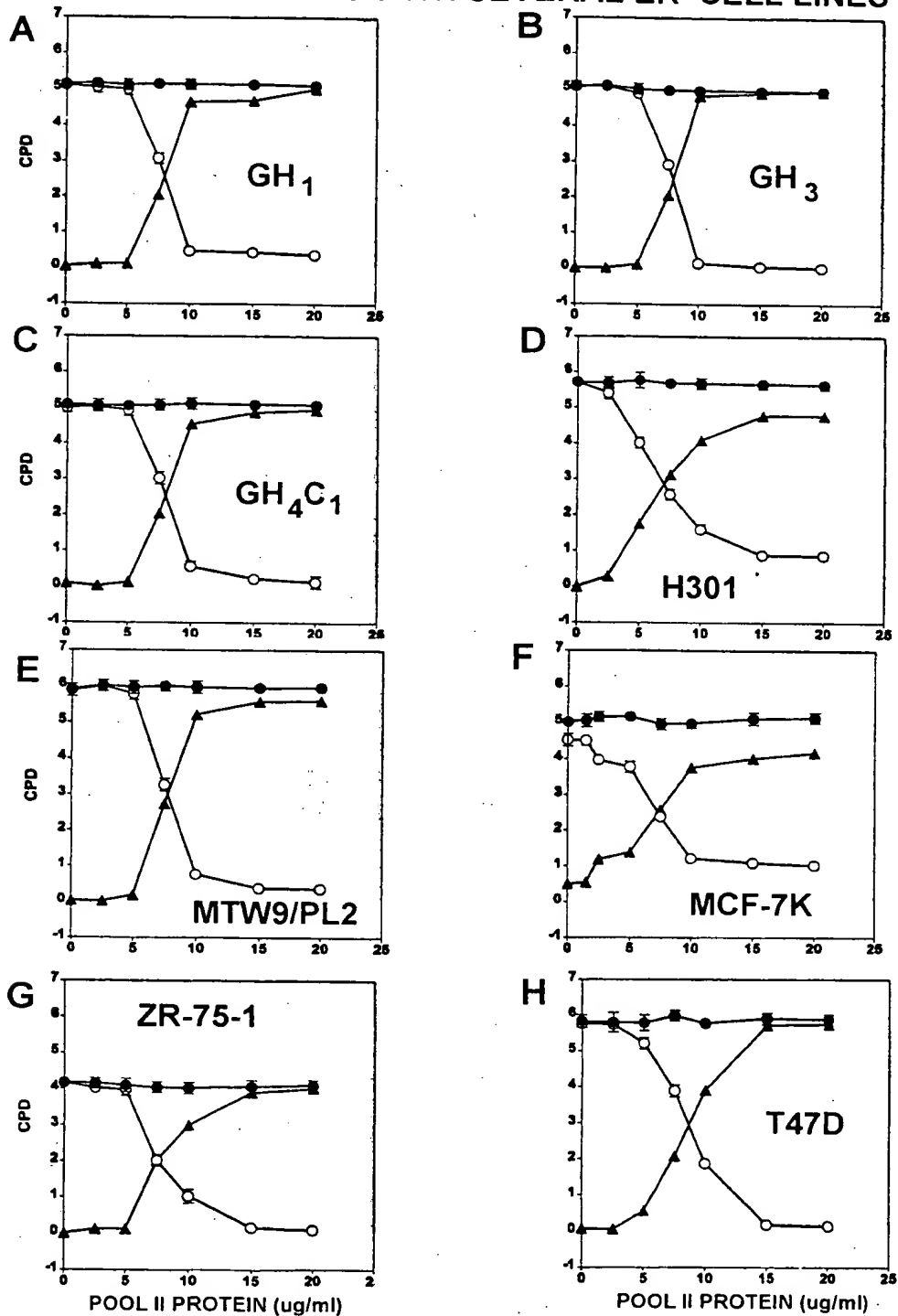
**SDS PAGE (A) AND WESTERN ANALYSIS (B) OF THREE
PREPARATIONS OF CA-PS-POOL II VS HUMAN SHBG**



LANES 1, 2, AND 3 = 10 ug each of CA-PS-POOL II

LANE "SHBG" = 10 mg of purified protein

FIGURE 82
ASSAY OF CA-PS-POOL II ESTROGEN REVERSIBLE
INHIBITORY ACTIVITY WITH SEVERAL ER CELL LINES



LEGEND:

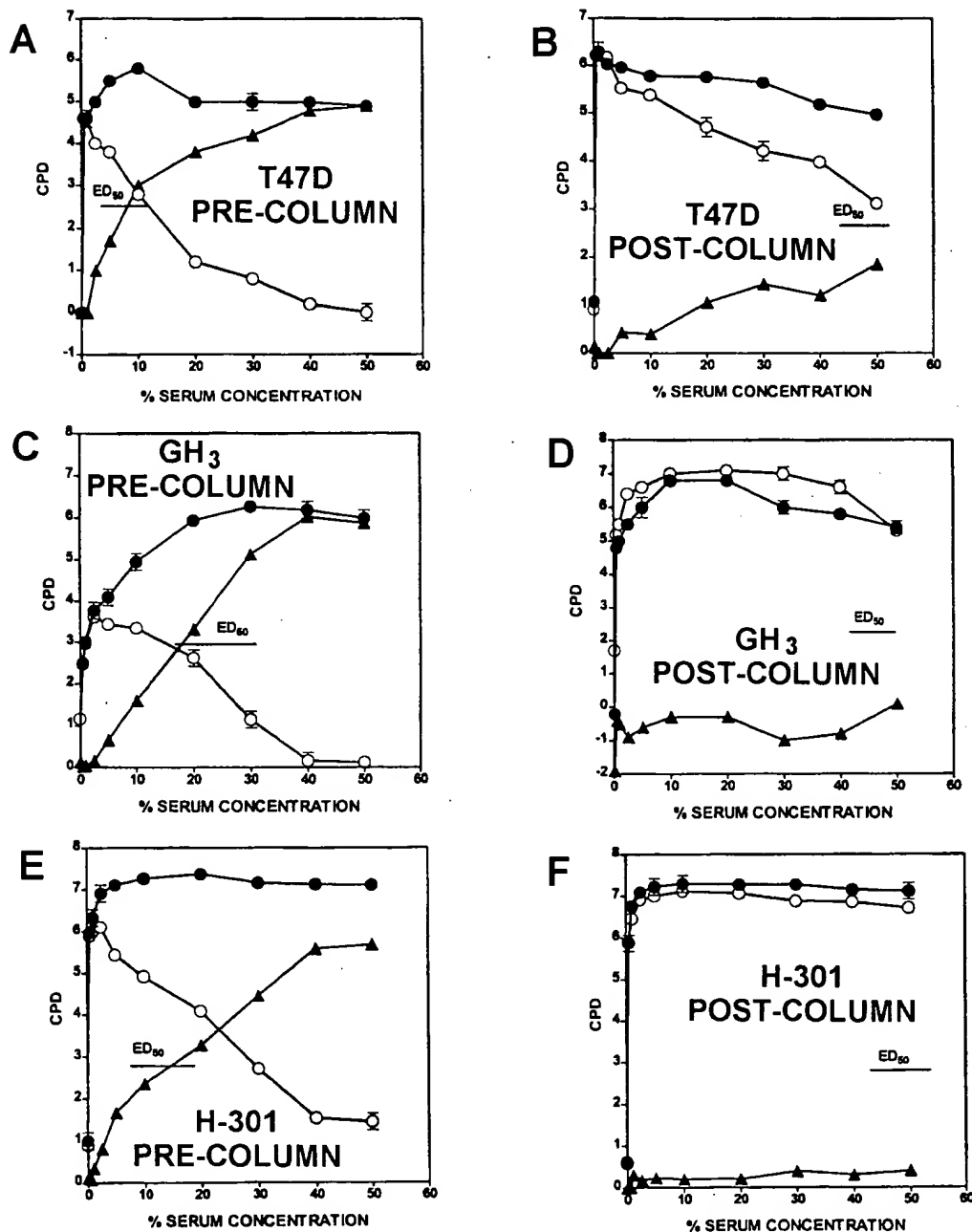
Open circles = - E₂

Closed circles = + E₂

Closed triangles = Estrogenic effect

FIGURE 83

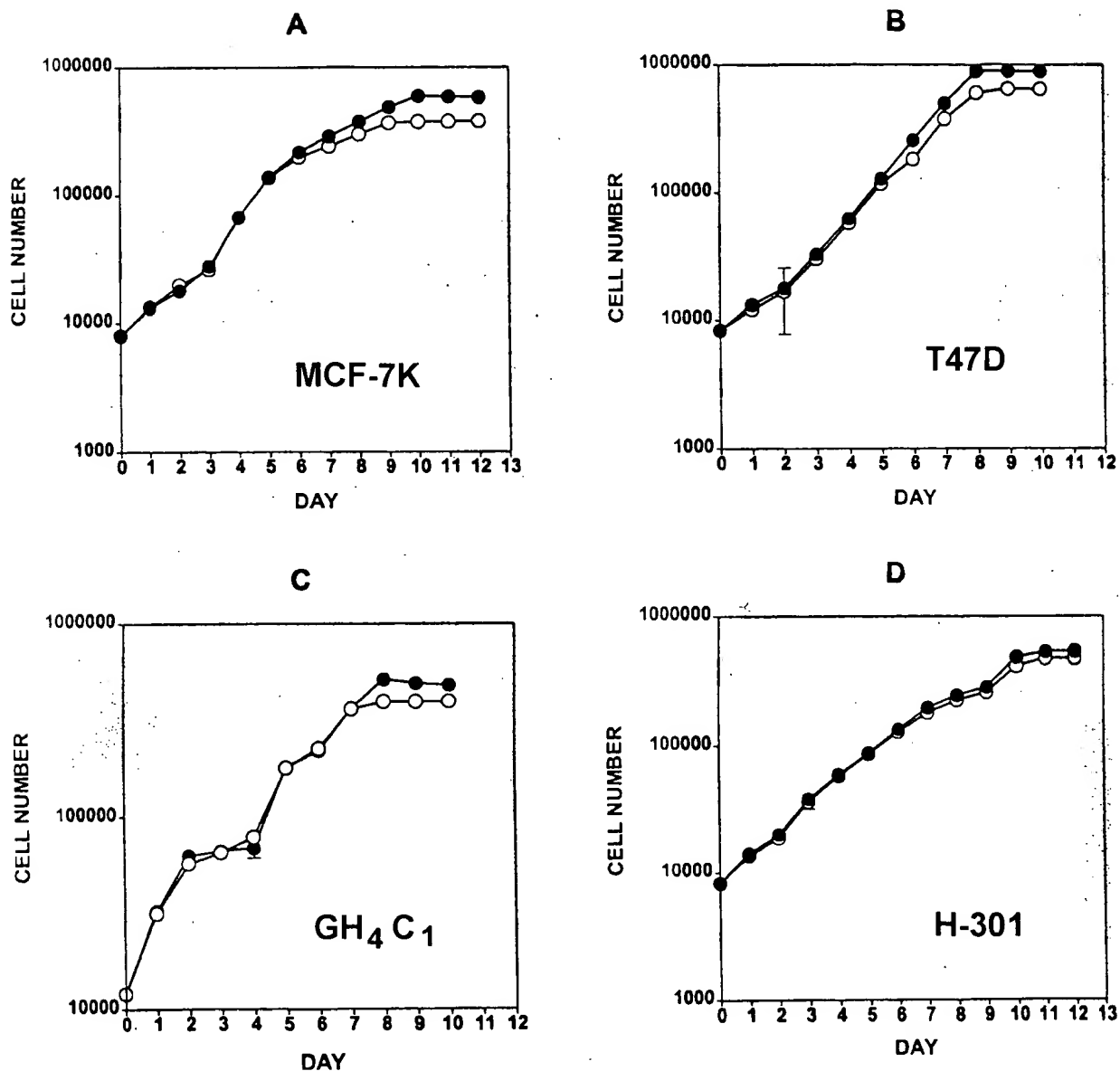
CORTISOL-AGAROSE AFFINITY REMOVAL OF THE INHIBITOR FROM CDE-SERUM



LEGEND: Open circles = - E₂
 Closed circles = + E₂
 Closed triangles = Estrogenic effect

FIGURE 84

**GROWTH OF ER⁺ CELL LINES IN
 SERUM-FREE MEDIUM \pm E₂**



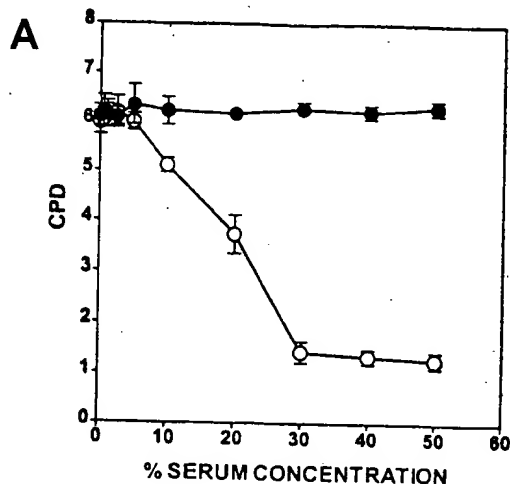
LEGEND:

Closed circles = + E₂

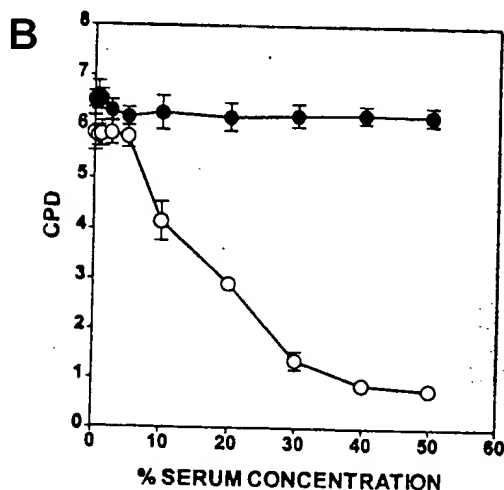
Open circles = - E₂

FIGURE 85

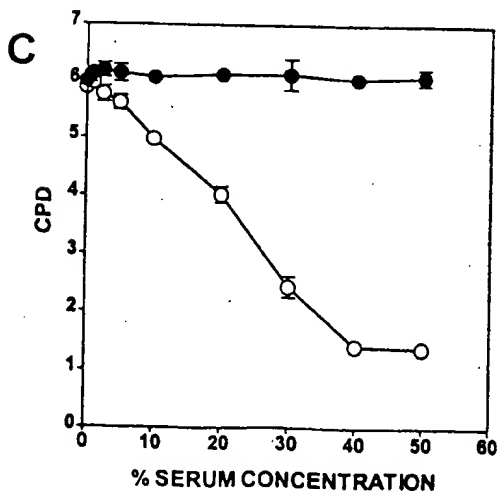
EFFECT OF CDE-SERUM ON ESTROGEN RESPONSIVE
GROWTH OF THREE ER⁺ CANCER CELL LINES IN SFM



A =
T47D IN DDM-2MF



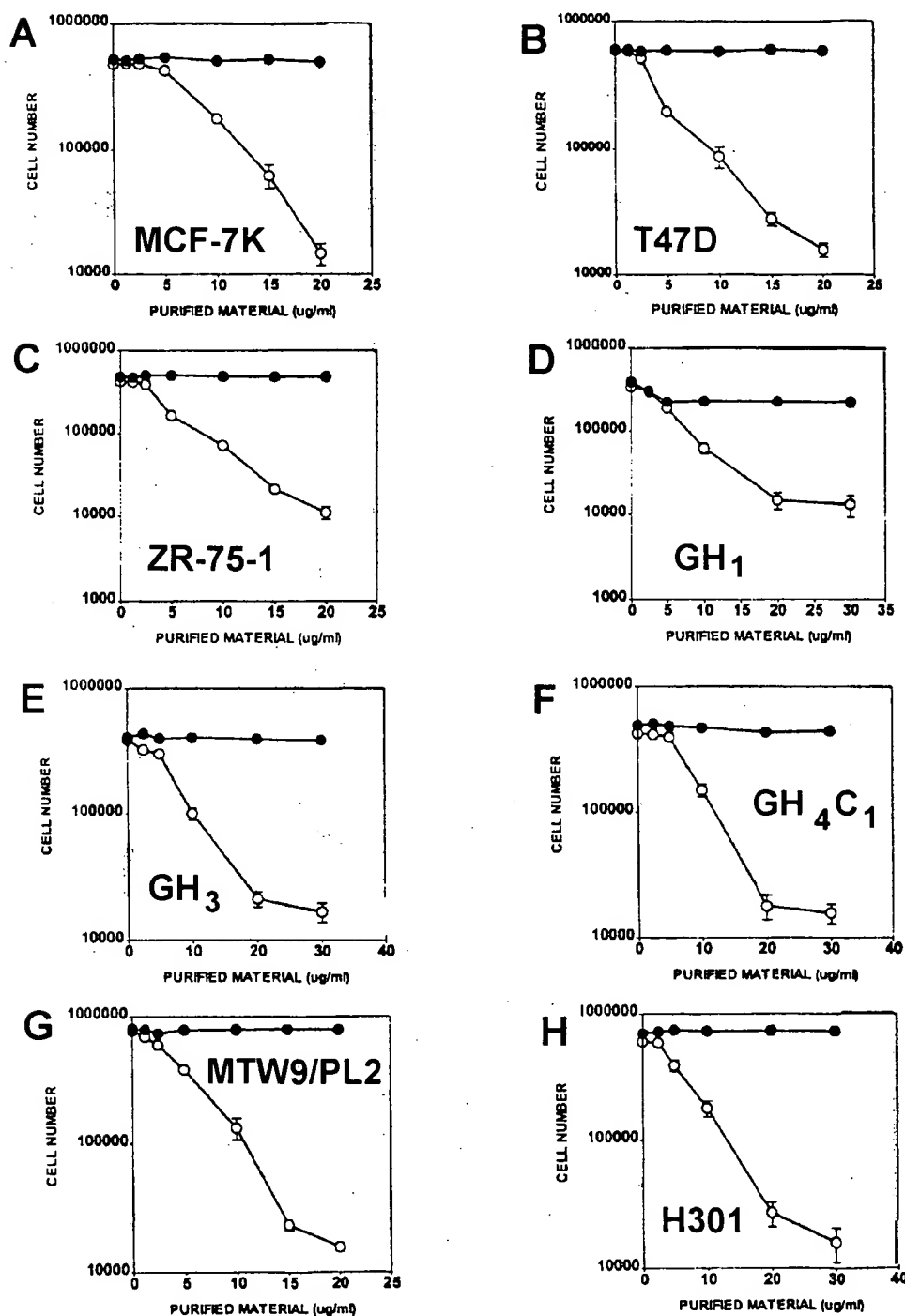
B =
MTW9/PL2 IN DDM-2A



C =
GH₄C₁ IN PCM 9

FIGURE 86

EFFECT OF CA-PS-POOL II ON ESTROGEN
 RESPONSIVE GROWTH IN SERUM FREE MEDIUM



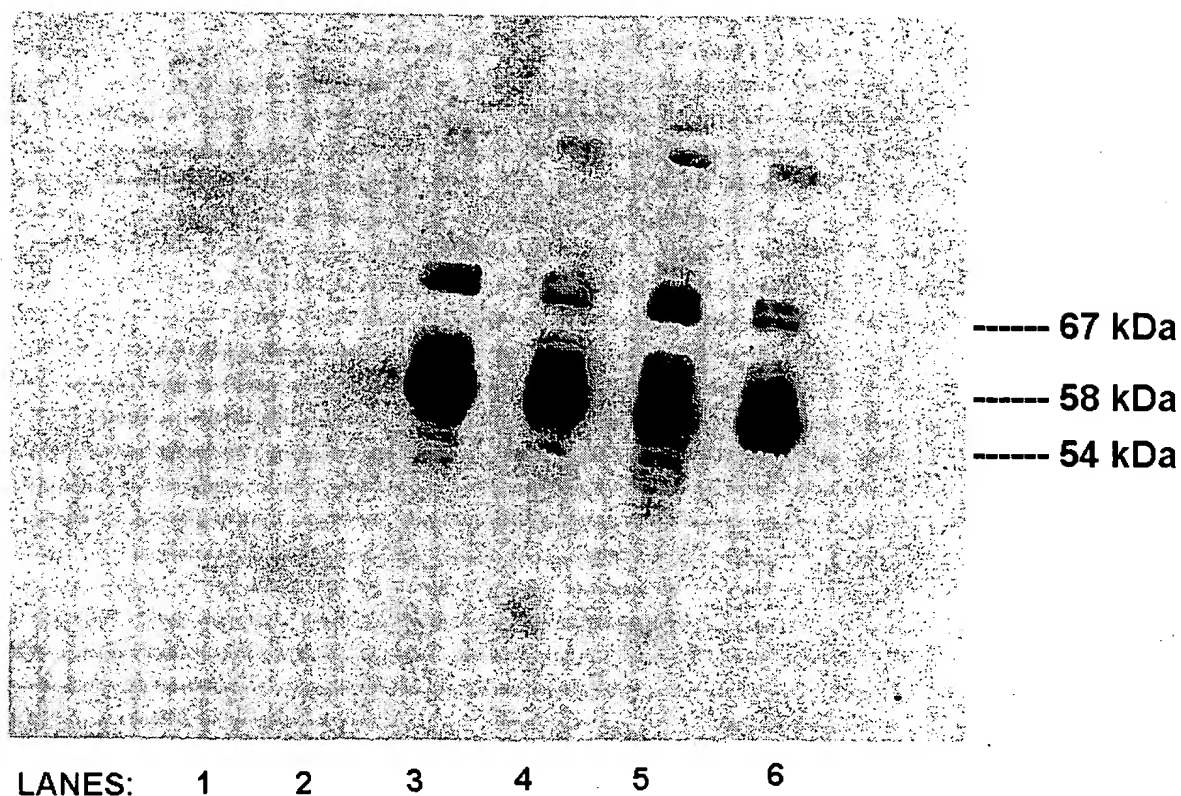
LEGEND: Open circles = $-E_2$
 Closed circles = $+E_2$

AMINO ACID SEQUENCING - HORSE SHBG

[illegible]

FIGURE 88

WESTERN ANALYSIS OF CBG (POOL I) AND SHBG (POOL II) PREPARATION WITH ANTI-54 kDa REST AVAILABLE COPY



1 = CBG PREPARATION # 5

2 = CBG PREPARATION # 6

3 = SHBG PREPARATION # 5.1

4 = SHBG PREPARATION # 5.2

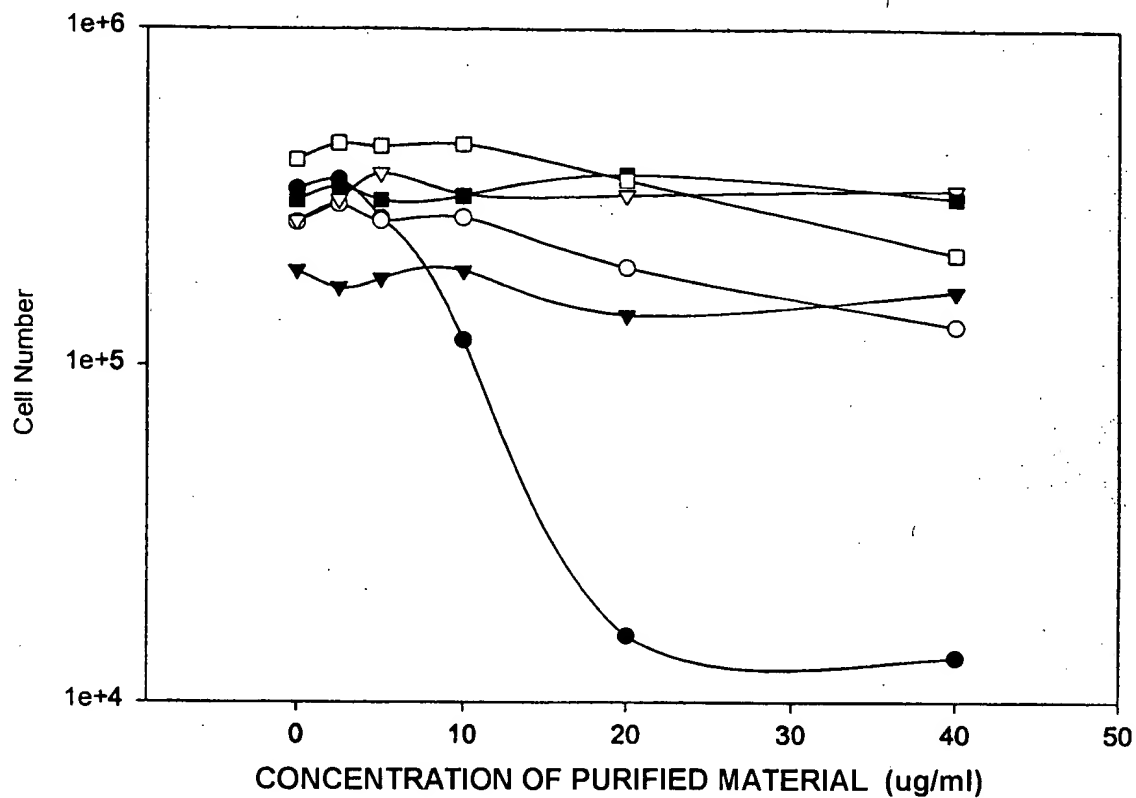
5 = SHBG PREPARATION # 6.1

6 = SHBG PREPARATION # 6.2

ANTIBODY = RABBIT ANTI-54 kDa 1:5000 DILUTION

FIGURE 89

EFFECT OF ANTI-54kDa ANTISERUM ON MTW9/PL2
CELLS GROWN IN THE PRESENCE OF CA-PS-POOL II

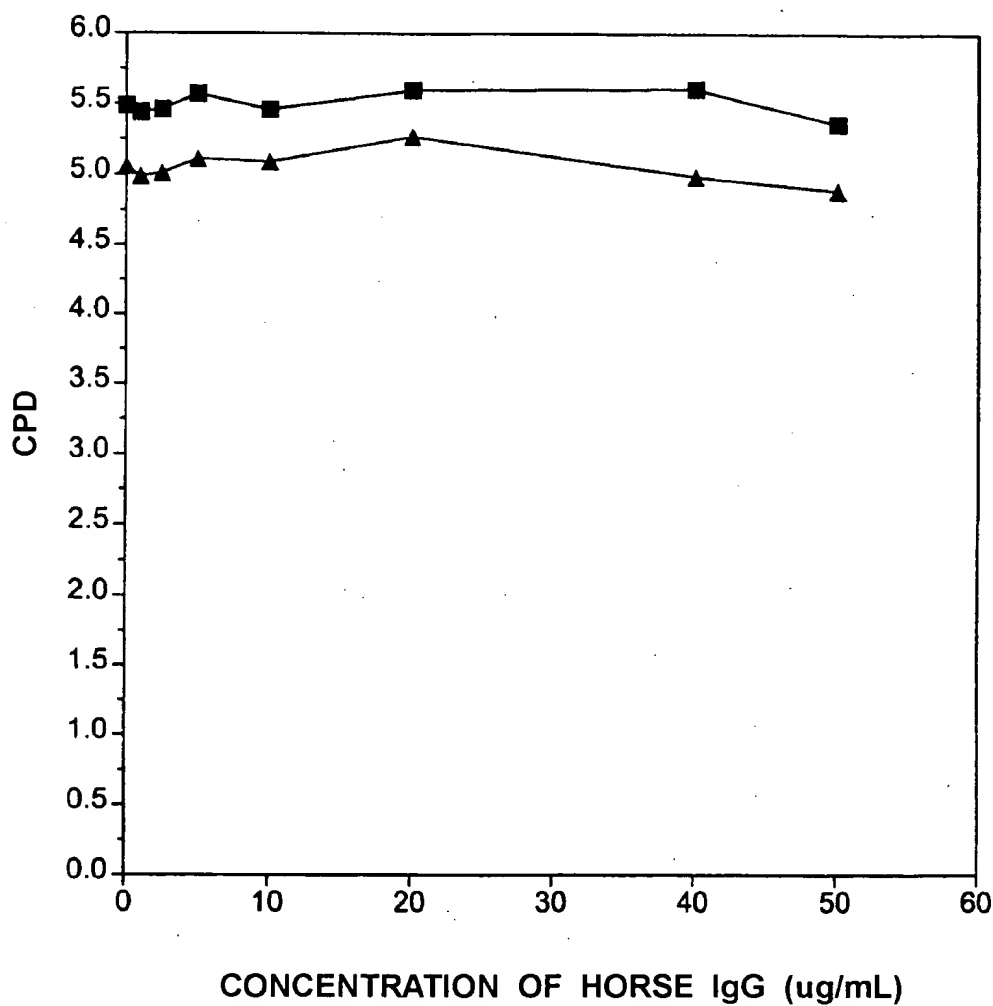


LEGEND:

- No antibody
- Antibody 1:5000
- ▼— Antibody 1:1000
- ▽— Antibody 1:500
- Antibody 1:100
- Antibody 1:50

FIGURE 91

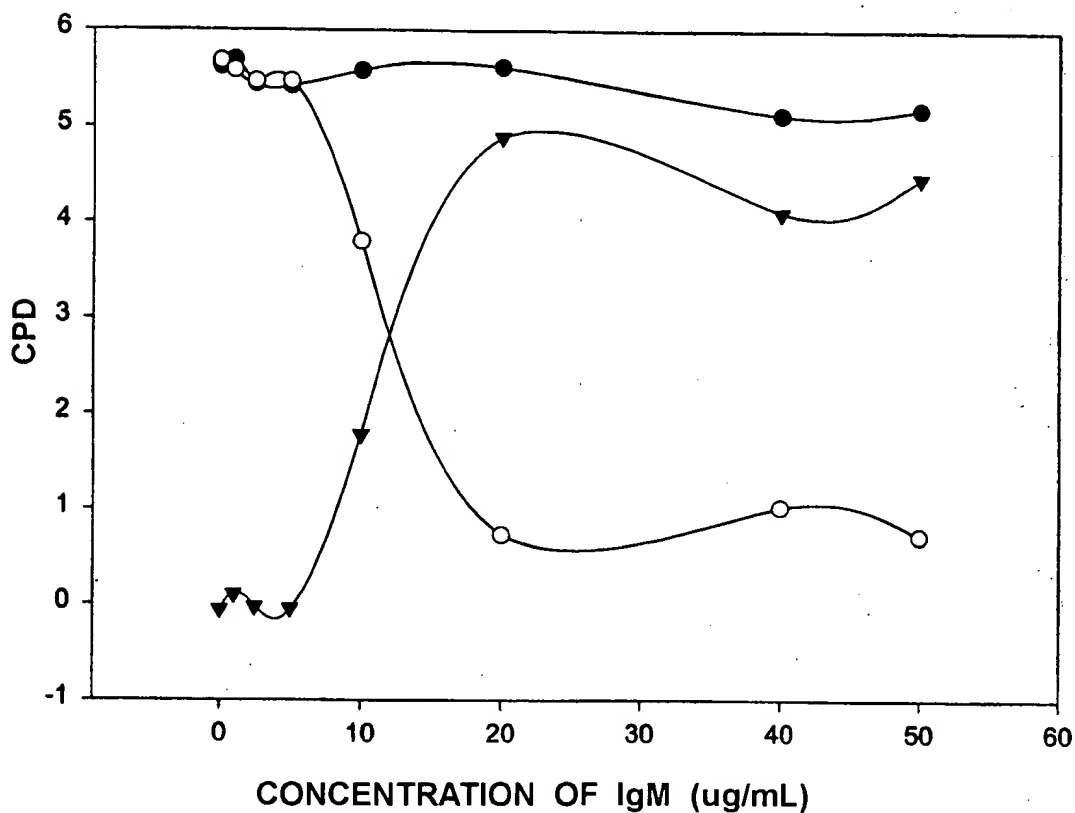
EFFECT OF COMMERCIALY PURIFIED HORSE IgG
ON MTW9/PL2 CELL GROWTH IN 2.5% CDE-HORSE SERUM



LEGEND: —■— plus E₂
 —▲— minus E₂

FIGURE 92

EFFECT OF HORSE IgM ON GROWTH OF THE
MTW9/PL2 CELLS IN 2.5% CDE HORSE SERUM $\pm E_2$

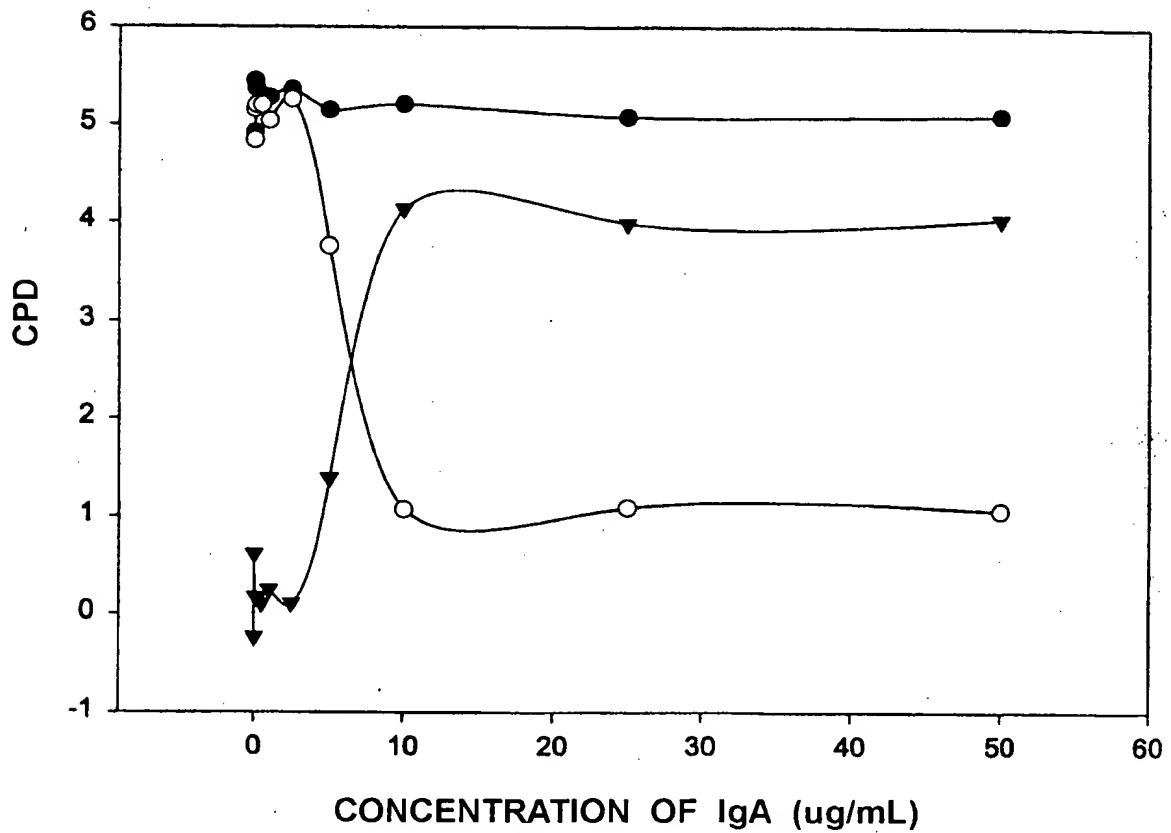


LEGEND:

- = + E_2
- = - E_2
- ▼— = Estrogenic effect

FIGURE 93

EFFECT OF HORSE IgA ON GROWTH OF THE
MTW9/PL2 CELLS IN 2.5% CDE HORSE SERUM $\pm E_2$

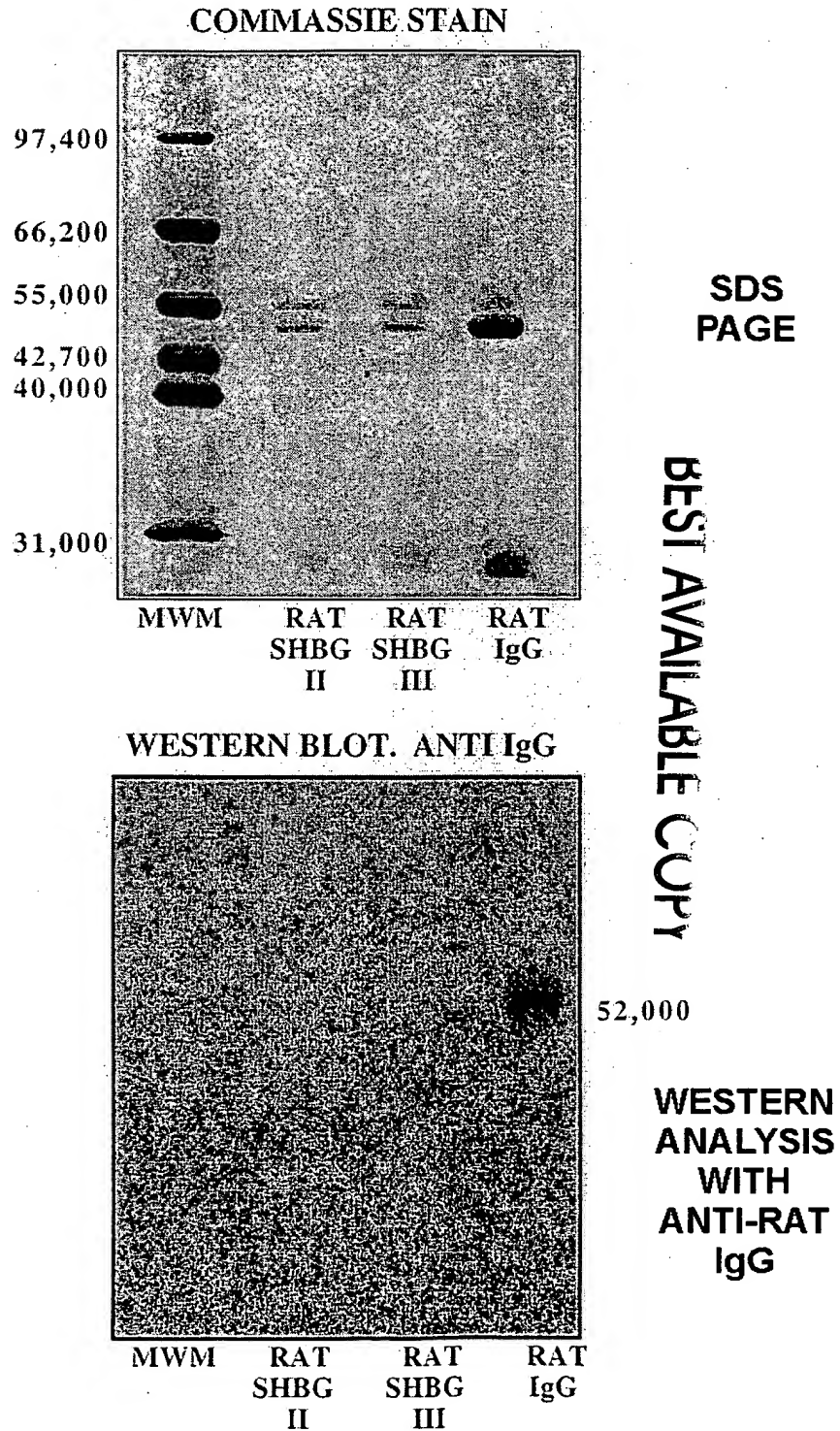


LEGEND:

- = + E_2
- = - E_2
- ▼ = Estrogenic effect

FIGURE 94

**SDS PAGE AND WESTERN ANALYSIS OF RAT
"SHBG-LIKE" PREPARATIONS**



09852959 051001

CROSSREACTION OF THE PURIFIED RAT "SHBG-LIKE" PROTEINS WITH ANTI- IgA, IgG1 AND IgM MONOCLONAL ANTIBODIES



hmSHBG
rbSHBG
hsABP
rtABP

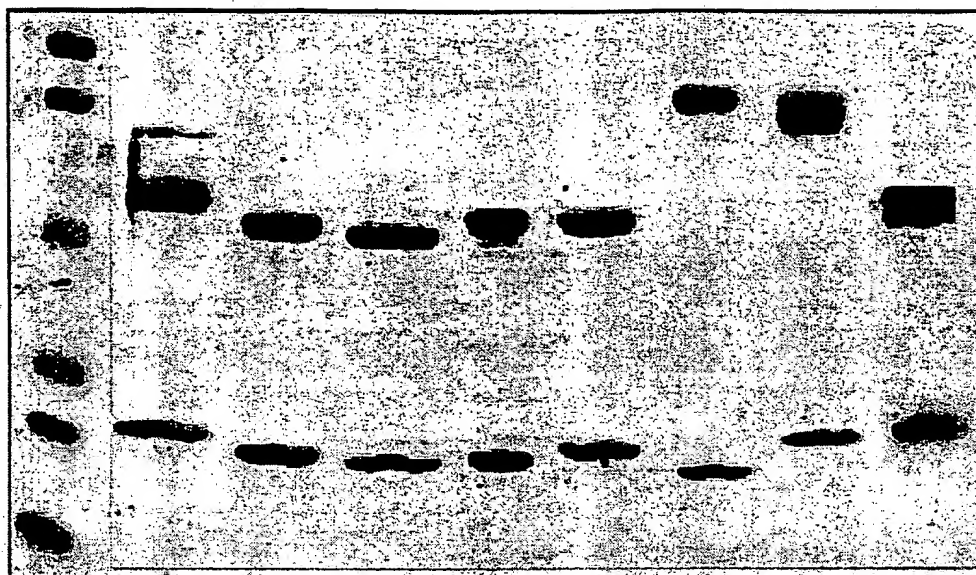
FIGURE 97

**SDS PAGE (A) AND WESTERN ANALYSIS (B)
 WITH ANTI-SHBG AND RAT Ig'S**

A KDa

RAT Igs COMMASSIE STAINED

104
82
46
33.4
28.3
19.4



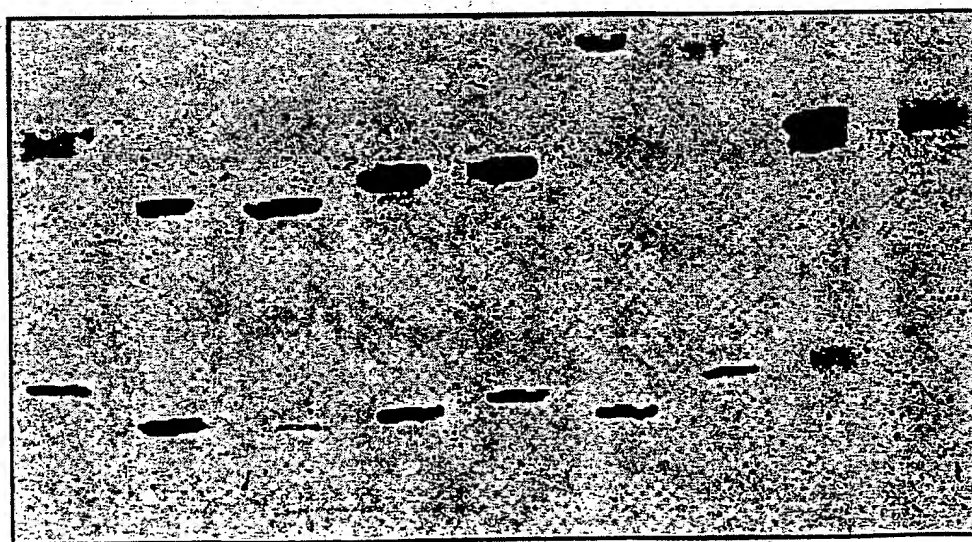
MW IgA IgG1 IgG2a IgG2b IgG2c IgE IgM RP

B

RAT Igs WESTERN BLOT. ANTI SHBG ANTIBODY

KDa

BEST AVAILABLE COPY

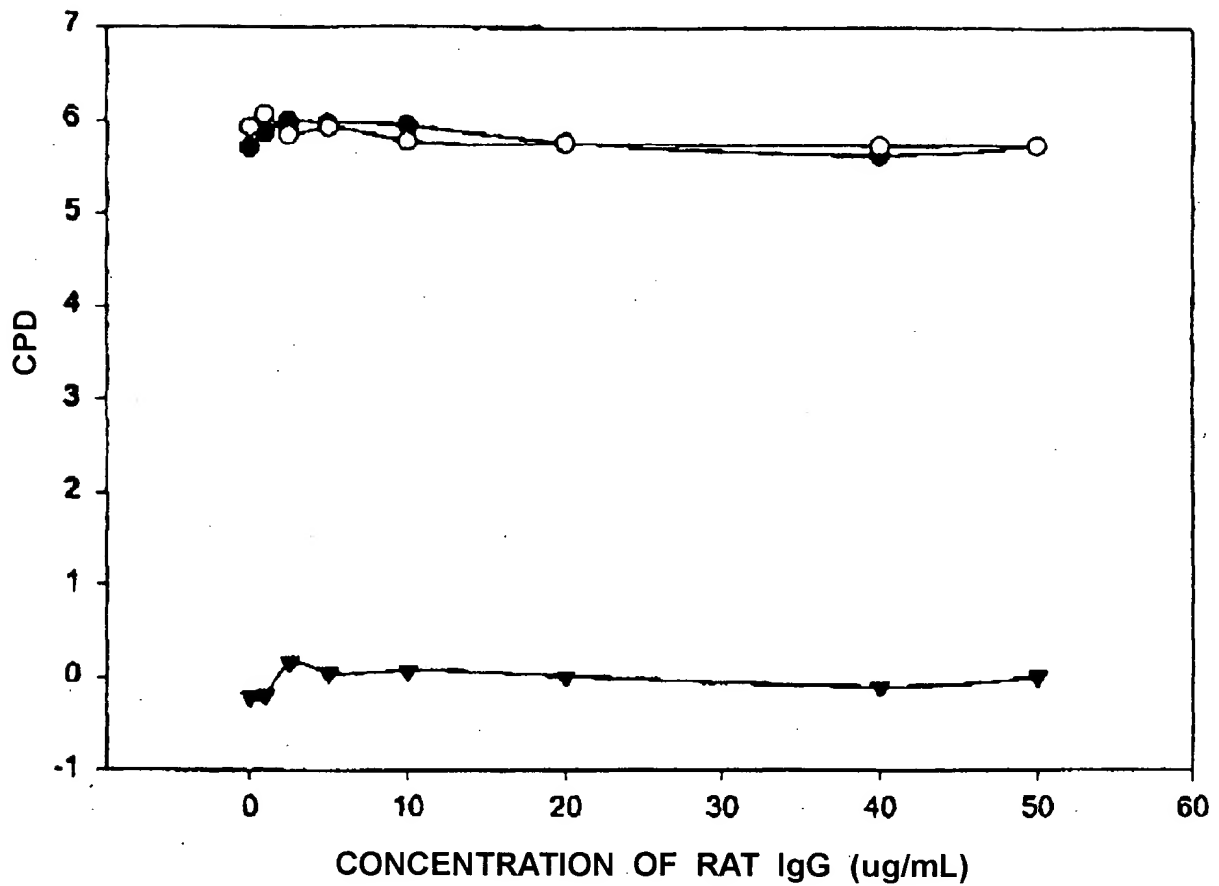


IgA IgG1 IgG2a IgG2b IgG2c IgE IgM HP RP

BEST AVAILABLE COPY

FIGURE 98

EFFECT OF RAT IgG ON MTW9/PL2 CELL
GROWTH IN 2.5% CDE RAT SERUM



LEGEND:

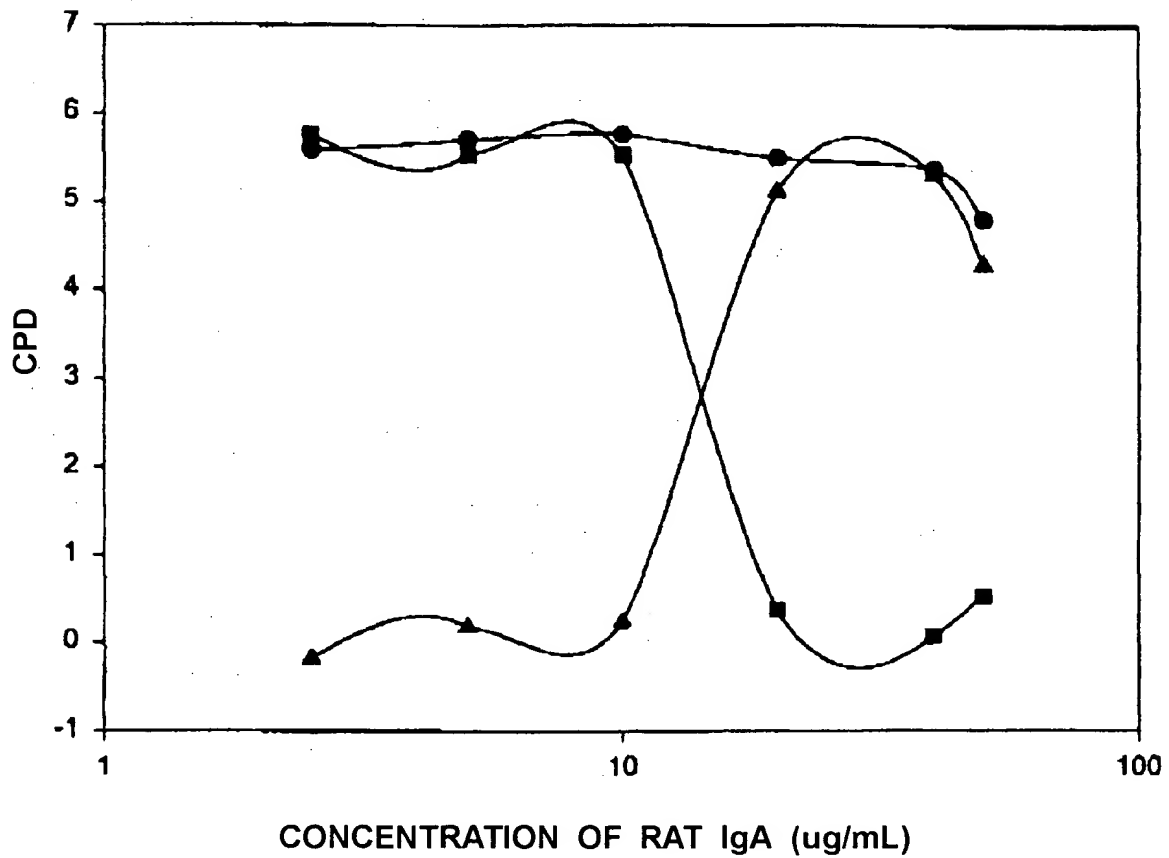
Closed circles = + E₂

Open circles = - E₂

Closed triangles = Estrogenic effect

FIGURE 99

**EFFECT OF RAT IgA ON MTW9/PL2 CELL
GROWTH IN 2.5% CDE RAT SERUM**



LEGEND:

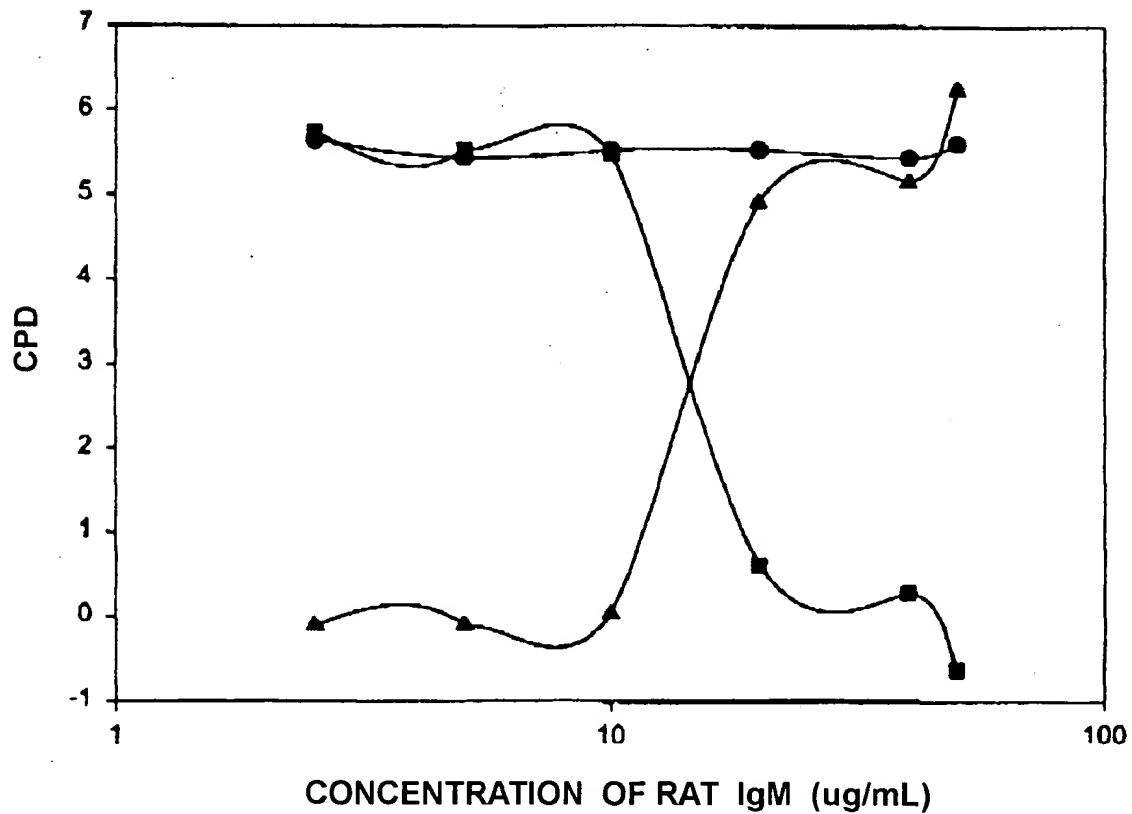
Closed circles = + E₂

Closed squares = - E₂

Closed triangles = Estrogenic effect

FIGURE 100

EFFECT OF RAT IgM ON MTW9/PL2 CELL
GROWTH IN 2.5% CDE RAT SERUM



LEGEND:

Closed squares = - E₂

Closed circles = + E₂

Closed triangles = Estrogenic effect

FIGURE 101

**ELUTION OF IgM FROM MANNAN
BINDING PROTEIN COLUMN**

BEST AVAILABLE COPY

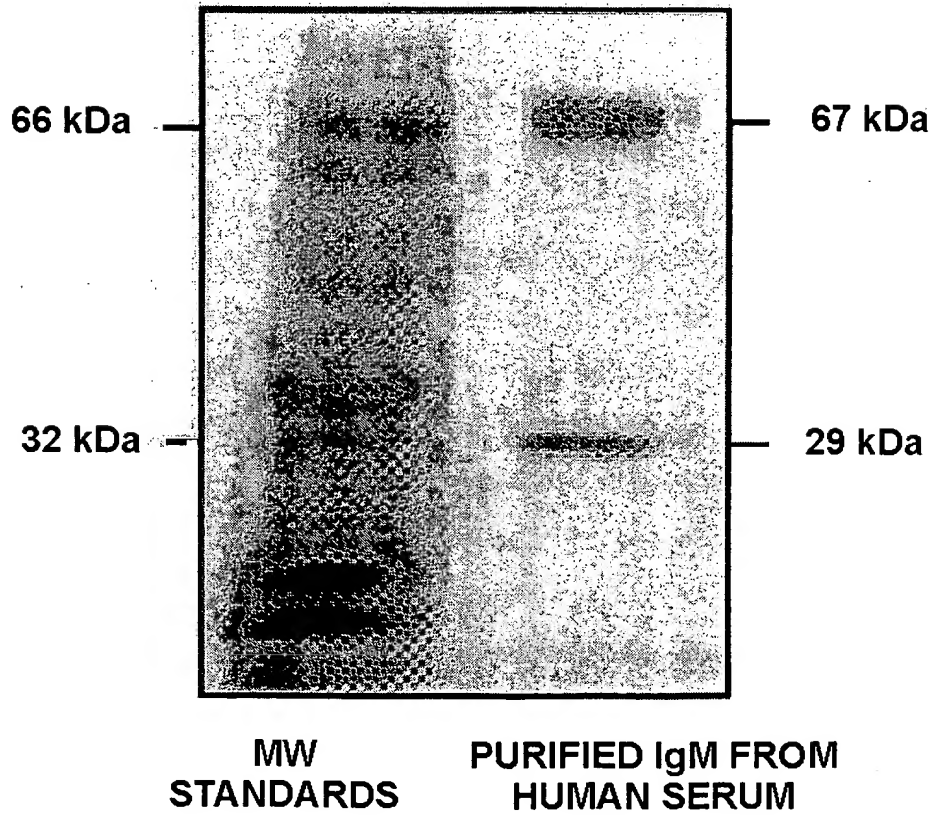
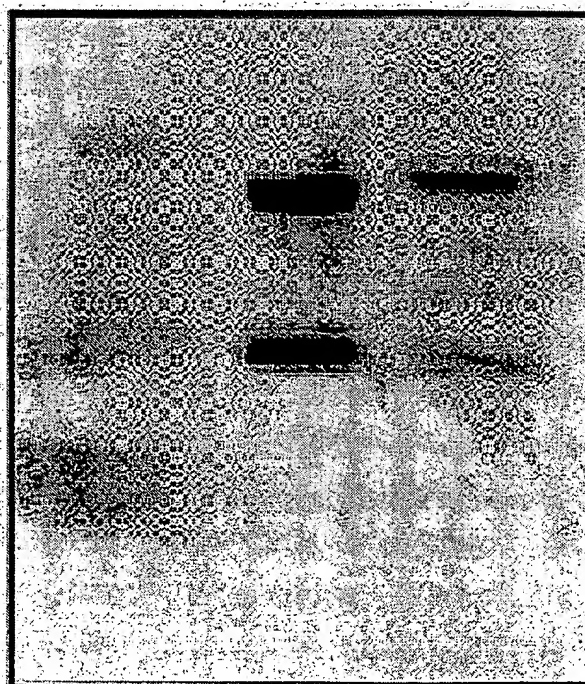


FIGURE 102

**IgM PURIFICATION FROM
PLASMA BY JACALIN**

BEST AVAILABLE COPY



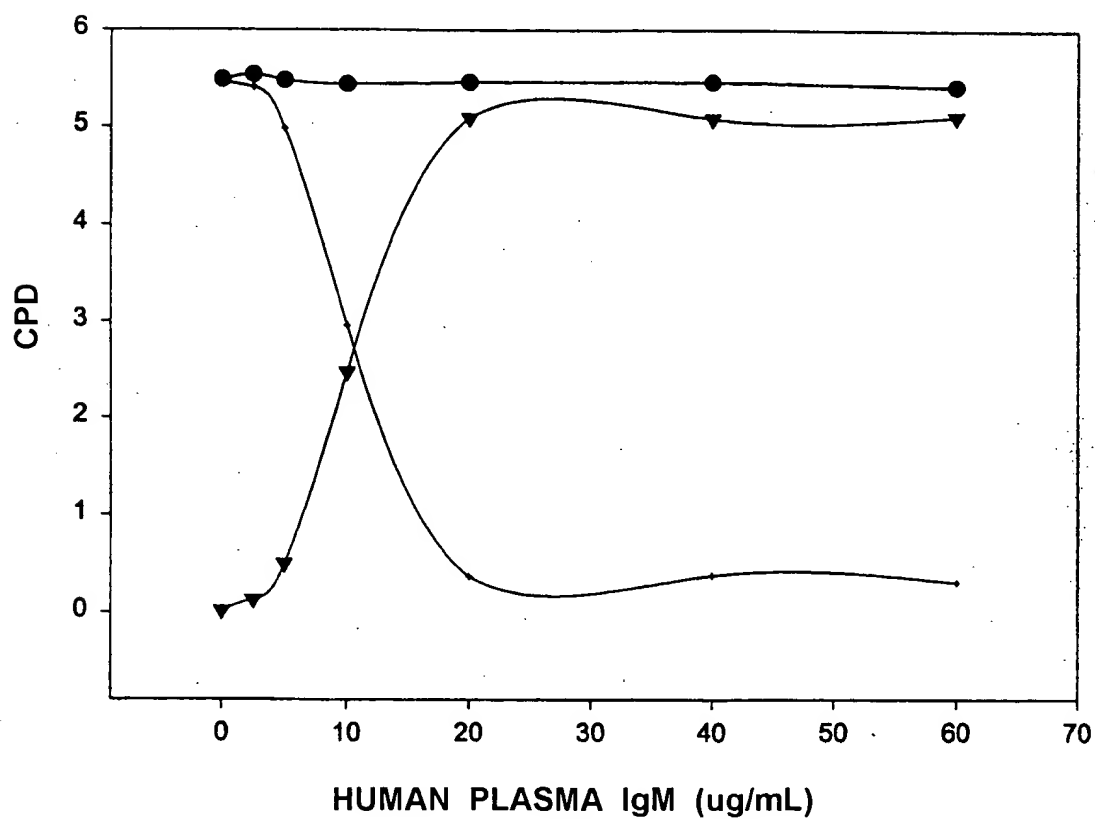
MW

**HUMAN
IgA**

**PURIFIED
IgA**

FIGURE 103

EFFECT OF IgM ISOLATED FROM HUMAN PLASMA
ON MTW9/PL2 GROWTH IN SERUM-FREE CONDITIONS



LEGEND:

- = + E₂
- = - E₂
- ▼ = Estrogenic effect

FIGURE 104

THE EFFECT OF VARIOUS IgA AND IgM PREPARATIONS
ON MTW9/PL2 CELLS GROWN IN SERUM-FREE MEDIUM

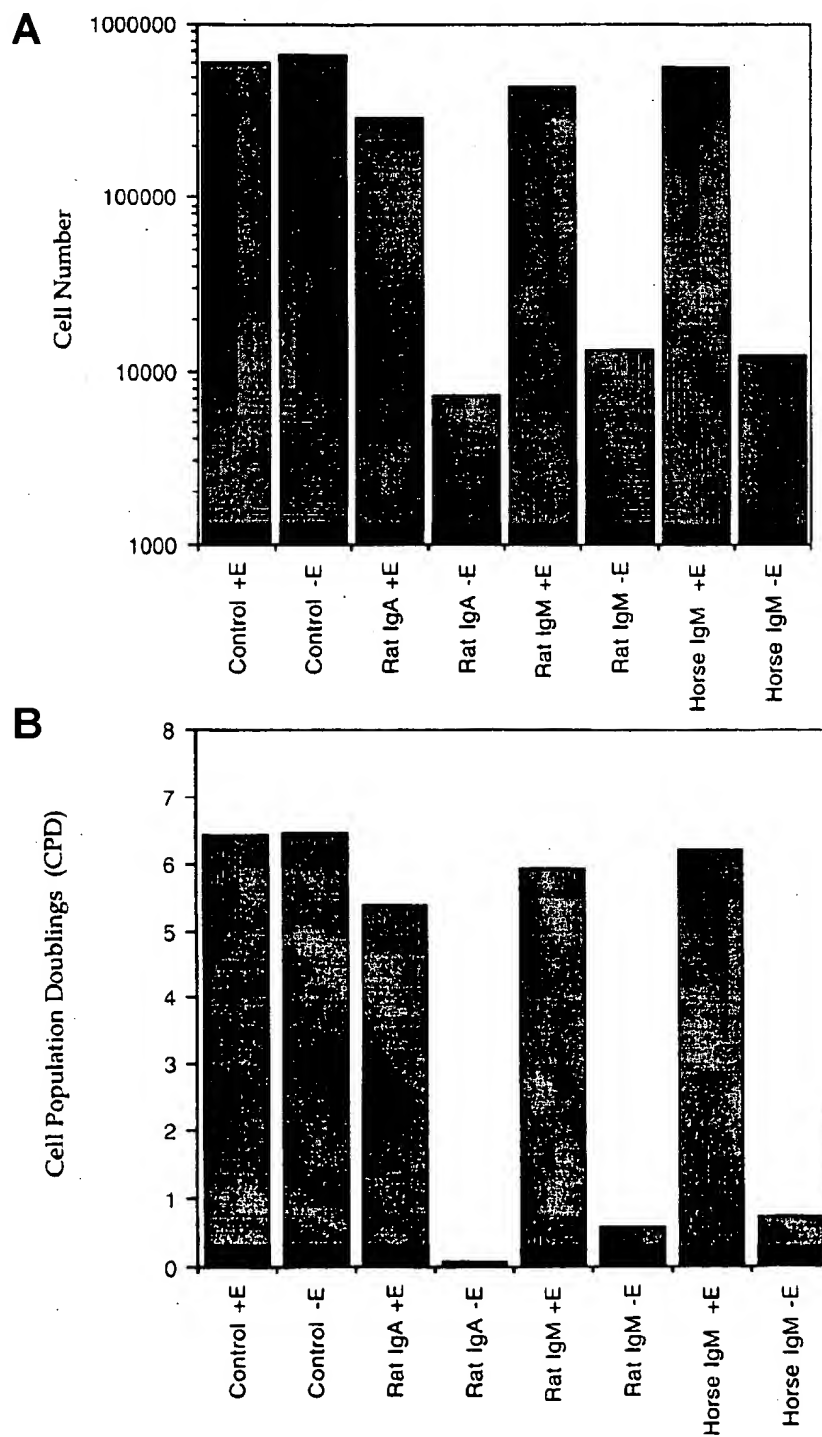
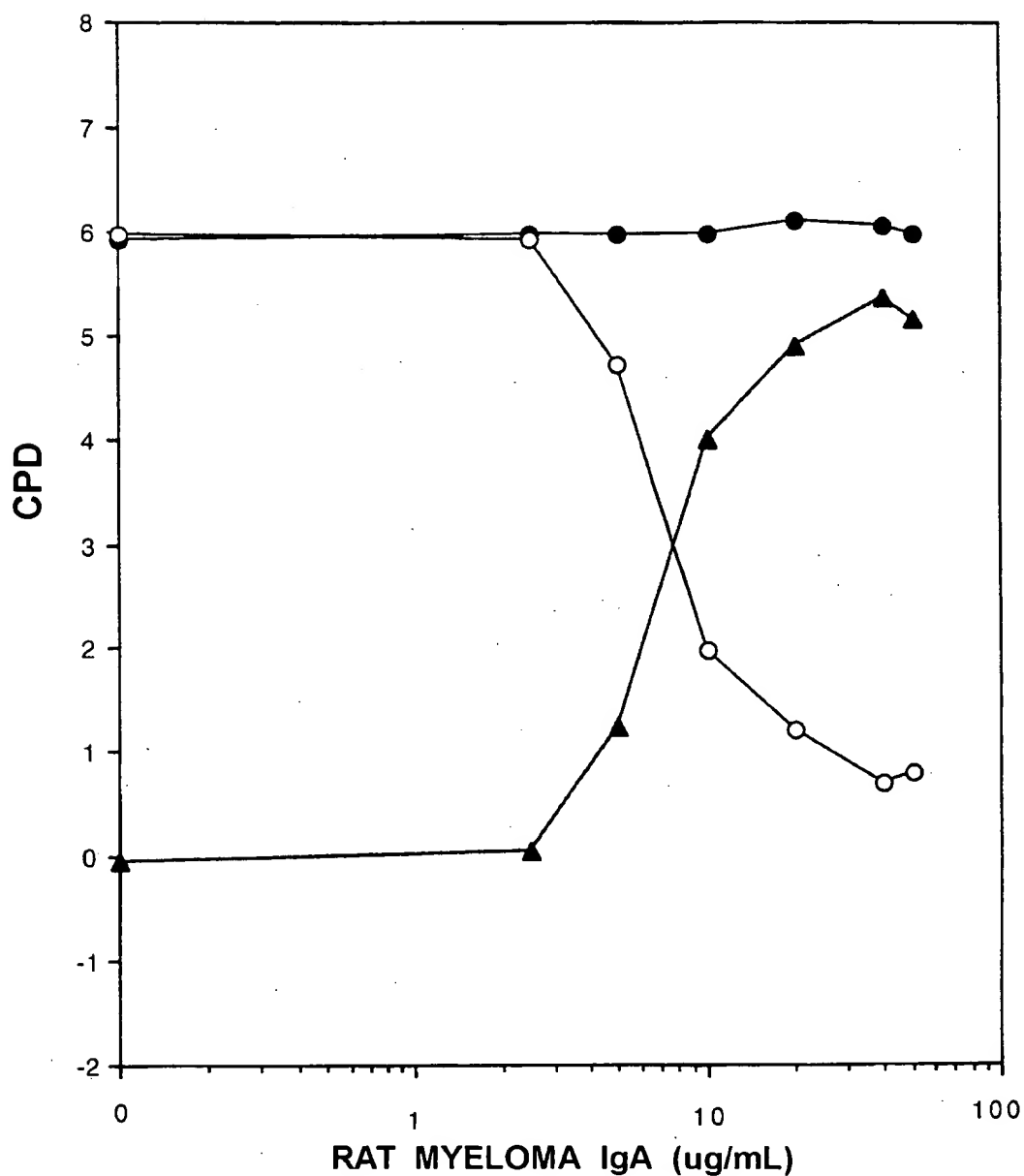


FIGURE 105

RAT MYELOMA IgA TITRATION ON GH₁ CELLS
GROWN IN SERUM-FREE CONDITIONS



LEGEND:

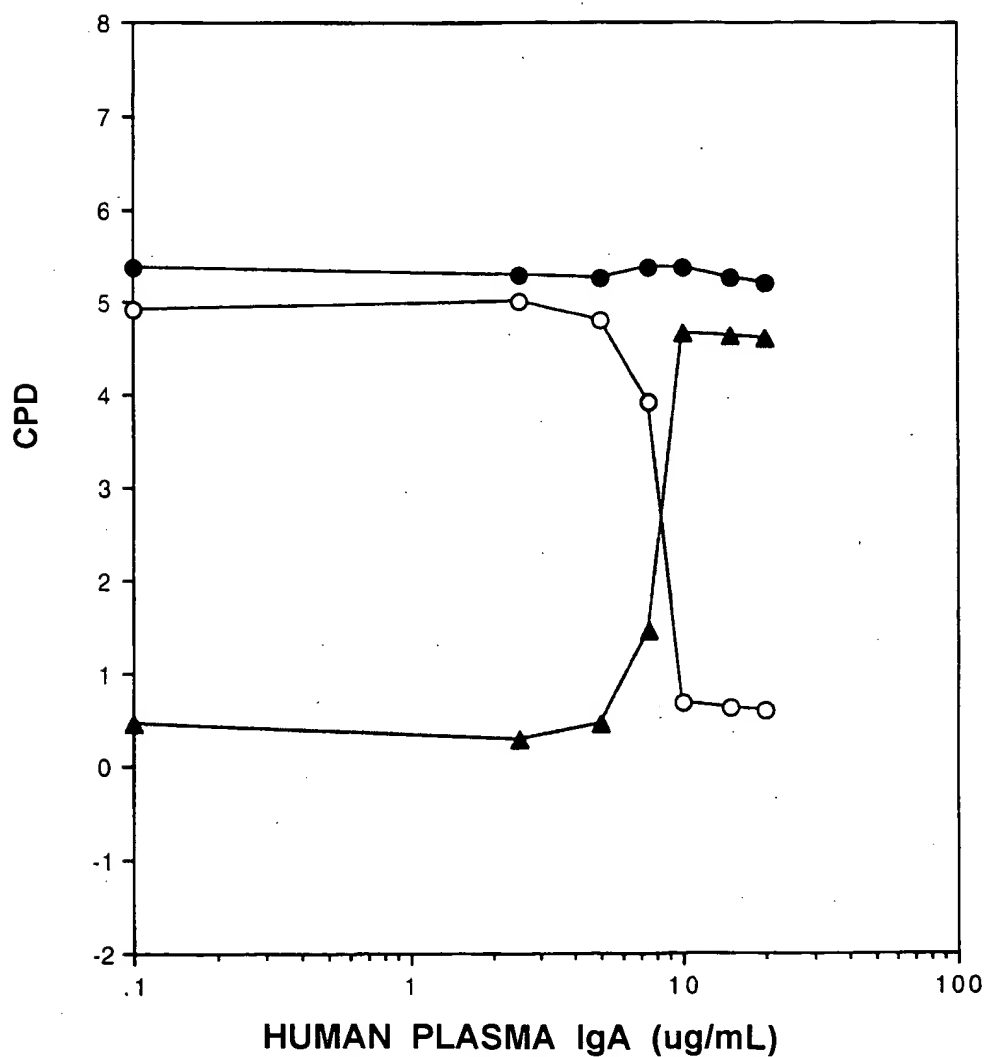
Closed circles = + E₂

Open circles = - E₂

Closed triangles = Estrogenic effect

FIGURE 106

HUMAN PLASMA IgA TITRATION ON GH₁ CELLS
GROWN IN SERUM-FREE CONDITIONS



LEGEND:

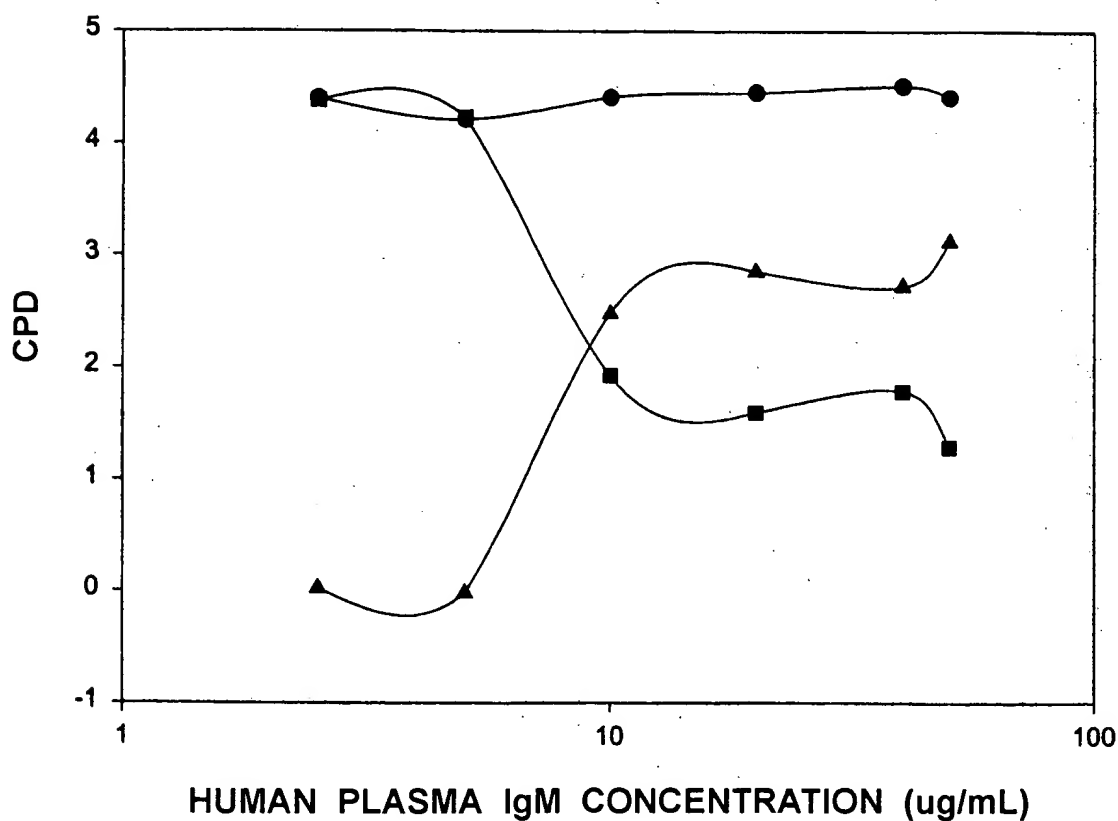
Closed circles = + E₂

Open circles = - E₂

Closed triangles = Estrogenic effect

FIGURE 107

HUMAN PLASMA IgM TITRATION ON GH₁ CELLS
GROWN IN SERUM-FREE CONDITIONS

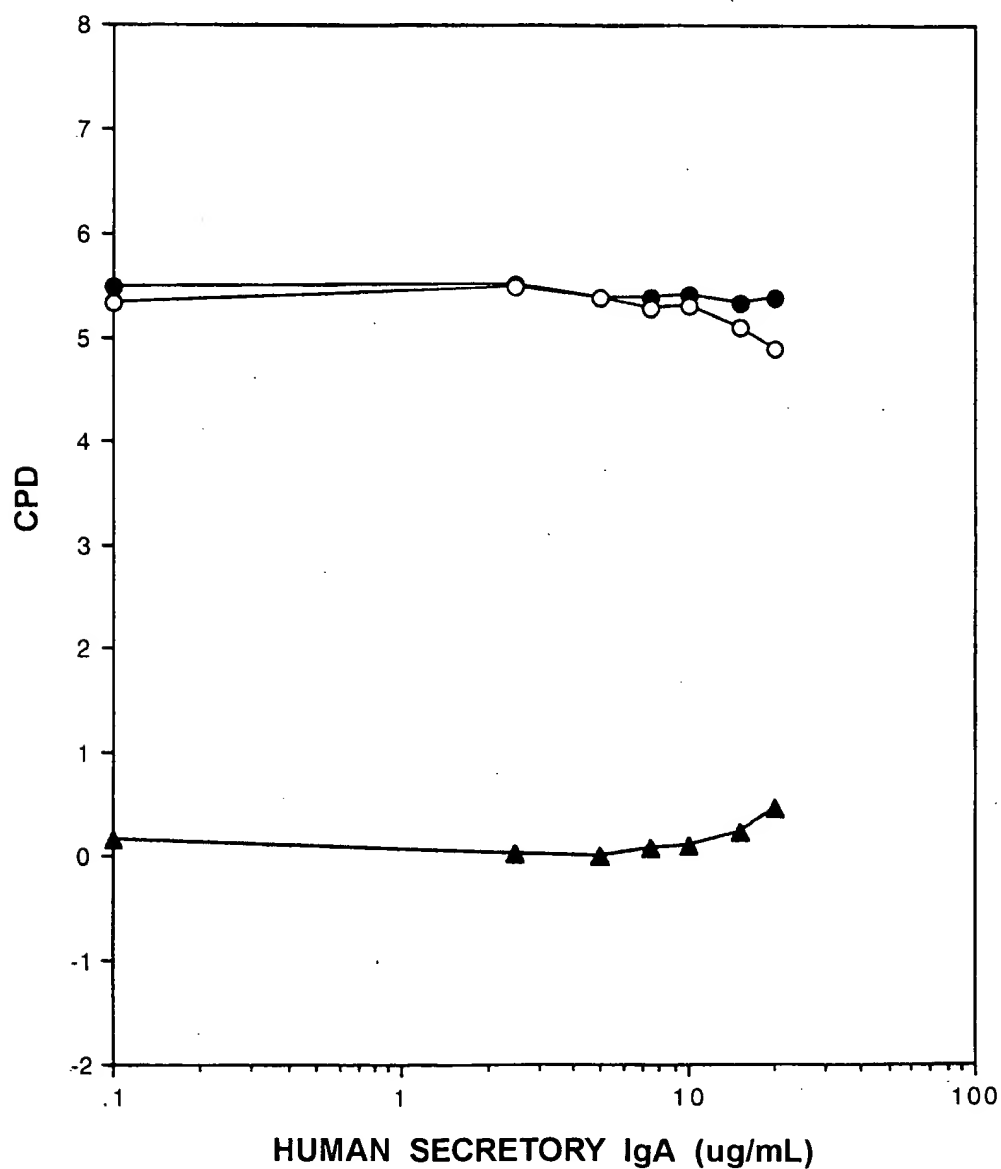


LEGEND:

- = + E₂
- = - E₂
- ▲— = Estrogenic effect

FIGURE 108

EFFECT OF HUMAN SECRETORY IgA ON
GH₁ CELLS GROWN IN SERUM-FREE CONDITIONS



LEGEND:

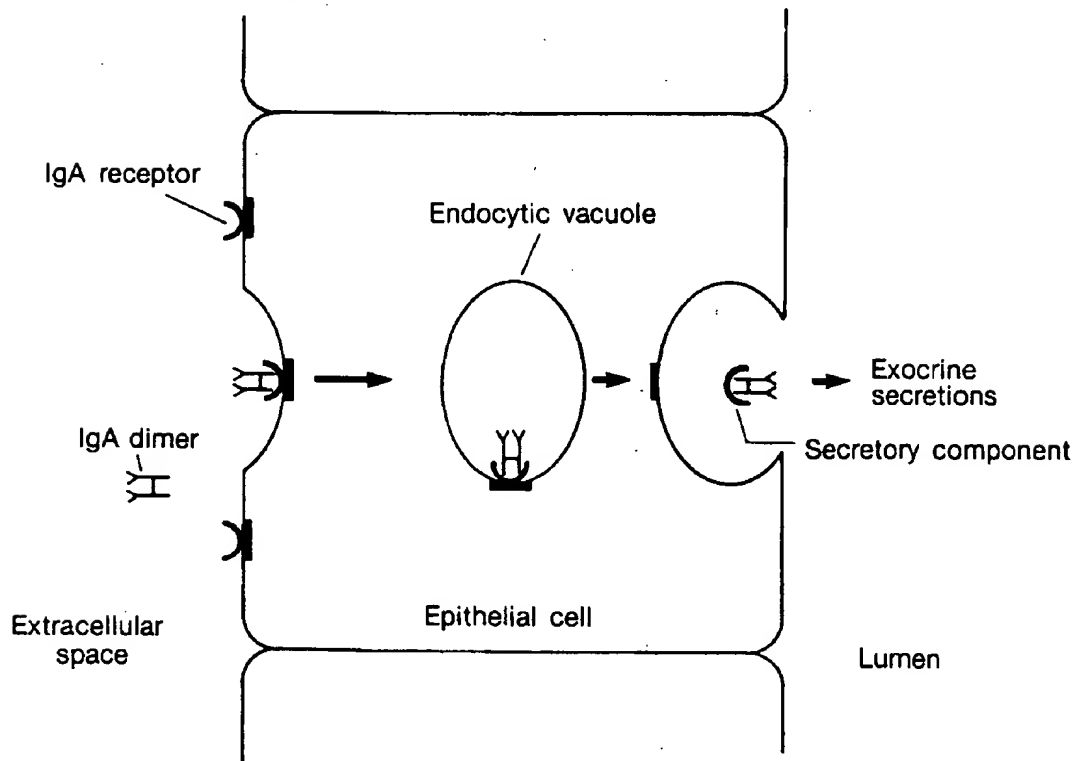
Closed circles = + E₂

Open circles = - E₂

Closed triangles = Estrogenic effect

FIGURE 109

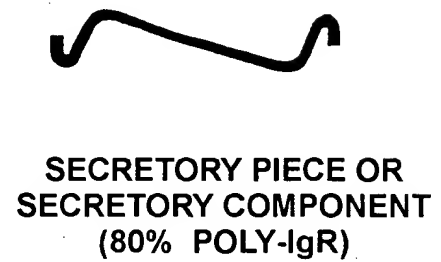
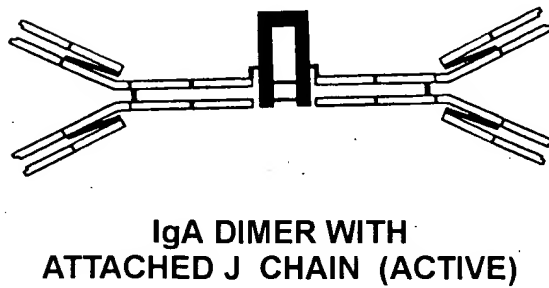
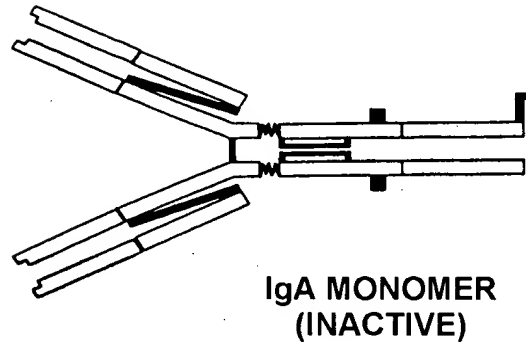
**MECHANISM OF TRANSCYTOSIS OF IgA AND IgM
BY MUCOSAL EPITHELIAL CELLS**



10050 3525360

FIGURE 110

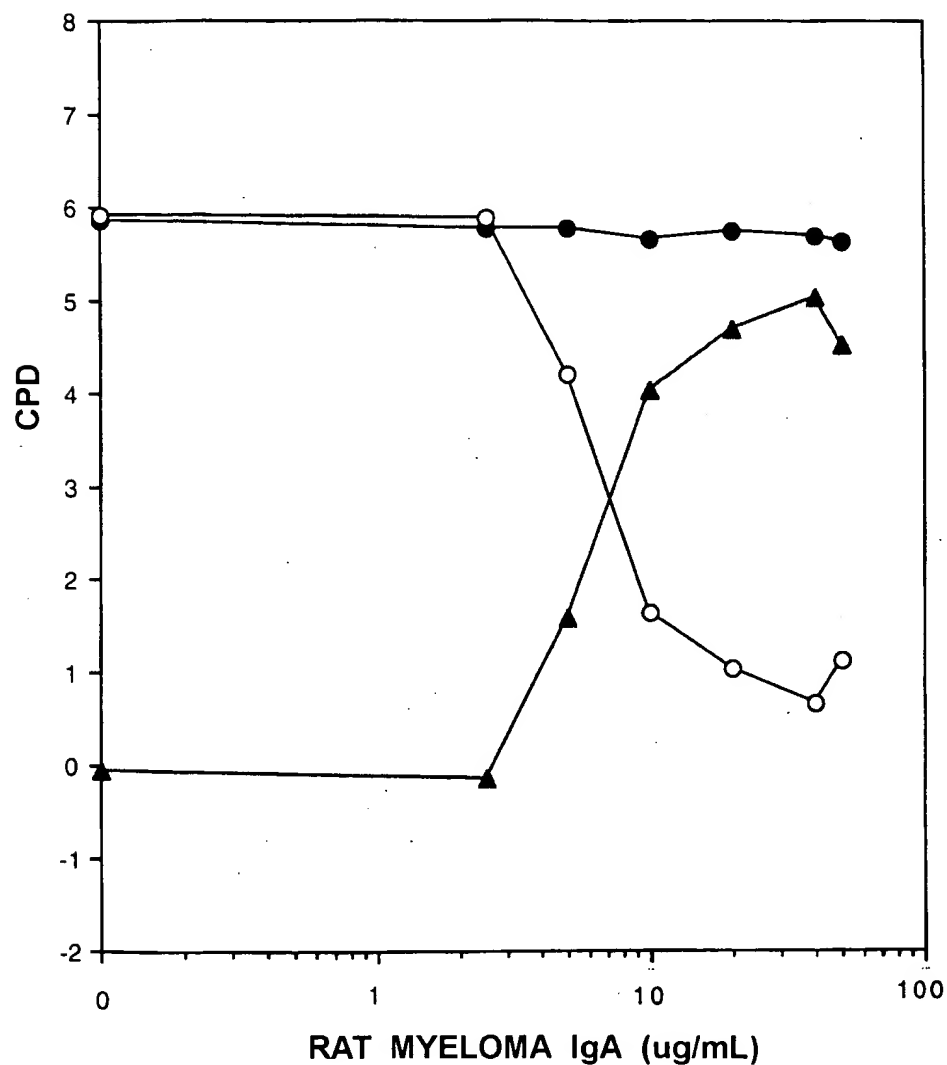
ESSENTIAL STRUCTURES OF HUMAN
PLASMA AND SECRETORY IgA



400450323900

FIGURE 111

EFFECT OF RAT MYELOMA IgA ON GH₃
CELLS GROWN IN SERUM-FREE MEDIUM



LEGEND:

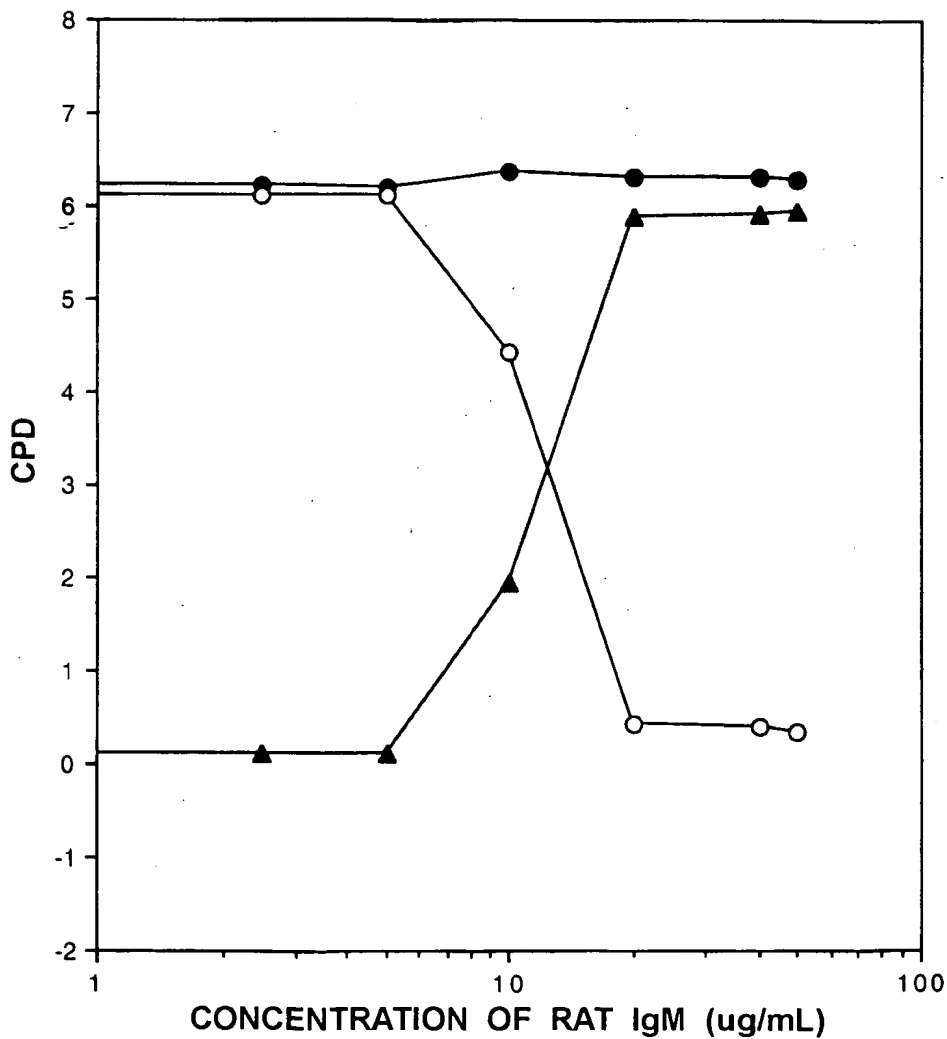
Closed circles = + E₂

Open circles = - E₂

Closed triangles = Estrogenic effect

FIGURE 112

EFFECT OF RAT IgM ON GH₃ CELL
GROWTH IN SERUM-FREE MEDIUM

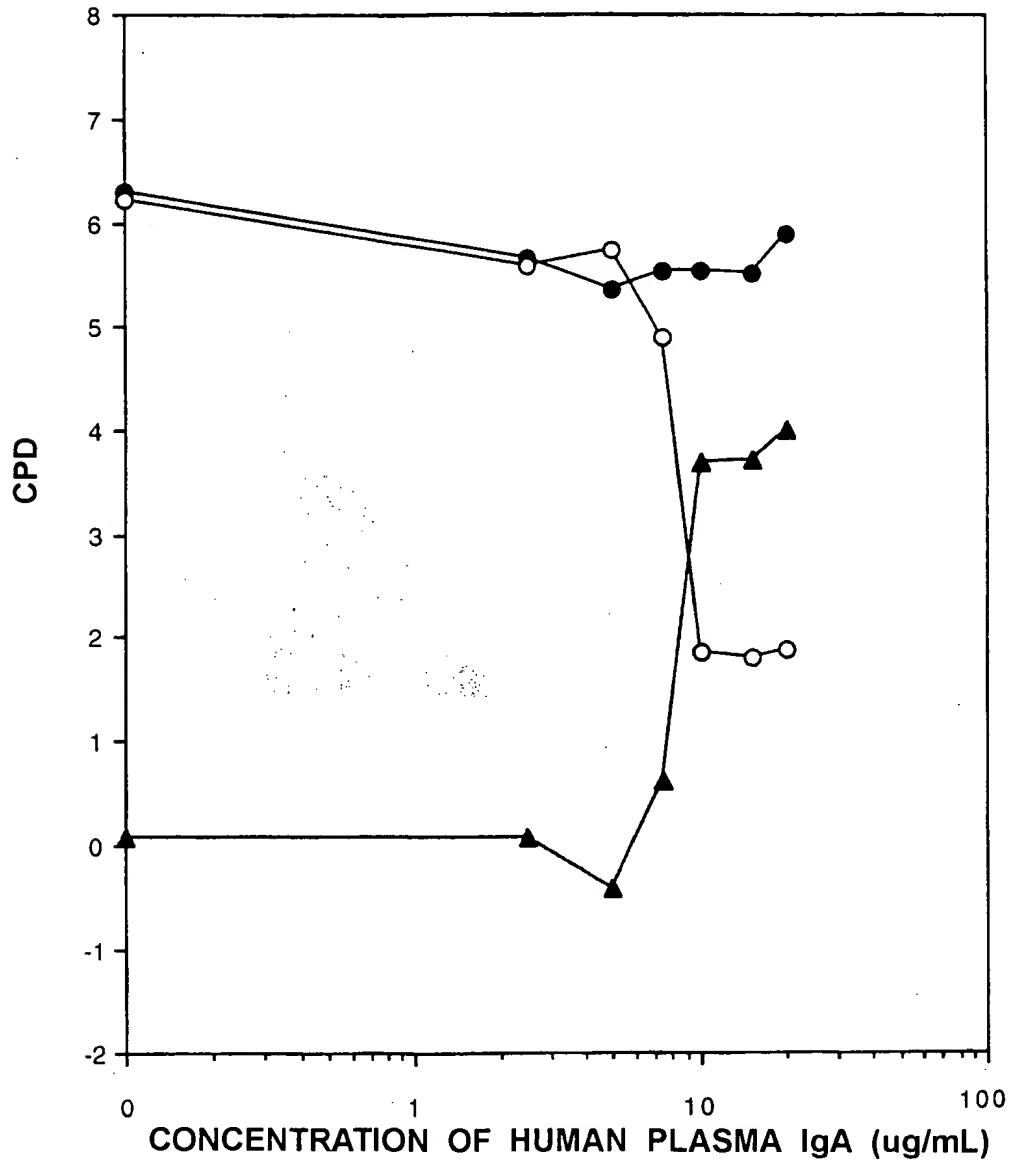


LEGEND:

- = + E₂
- = - E₂
- ▲— = Estrogenic effect

FIGURE 113

EFFECT OF HUMAN PLASMA IgA ON GH₃
CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

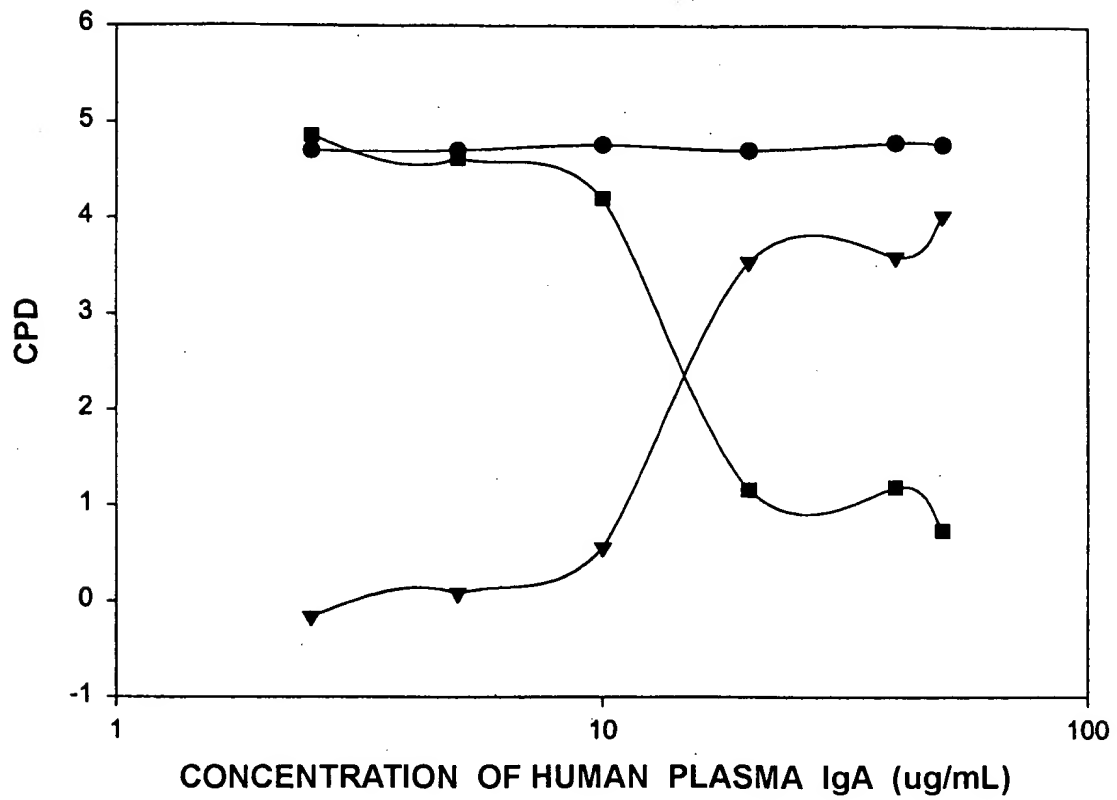
Closed circles = + E₂

Open circles = - E₂

Closed triangles = Estrogenic effect

FIGURE 114

EFFECT OF HUMAN PLASMA IgM ON GH₃
CELL GROWTH IN SERUM-FREE MEDIUM

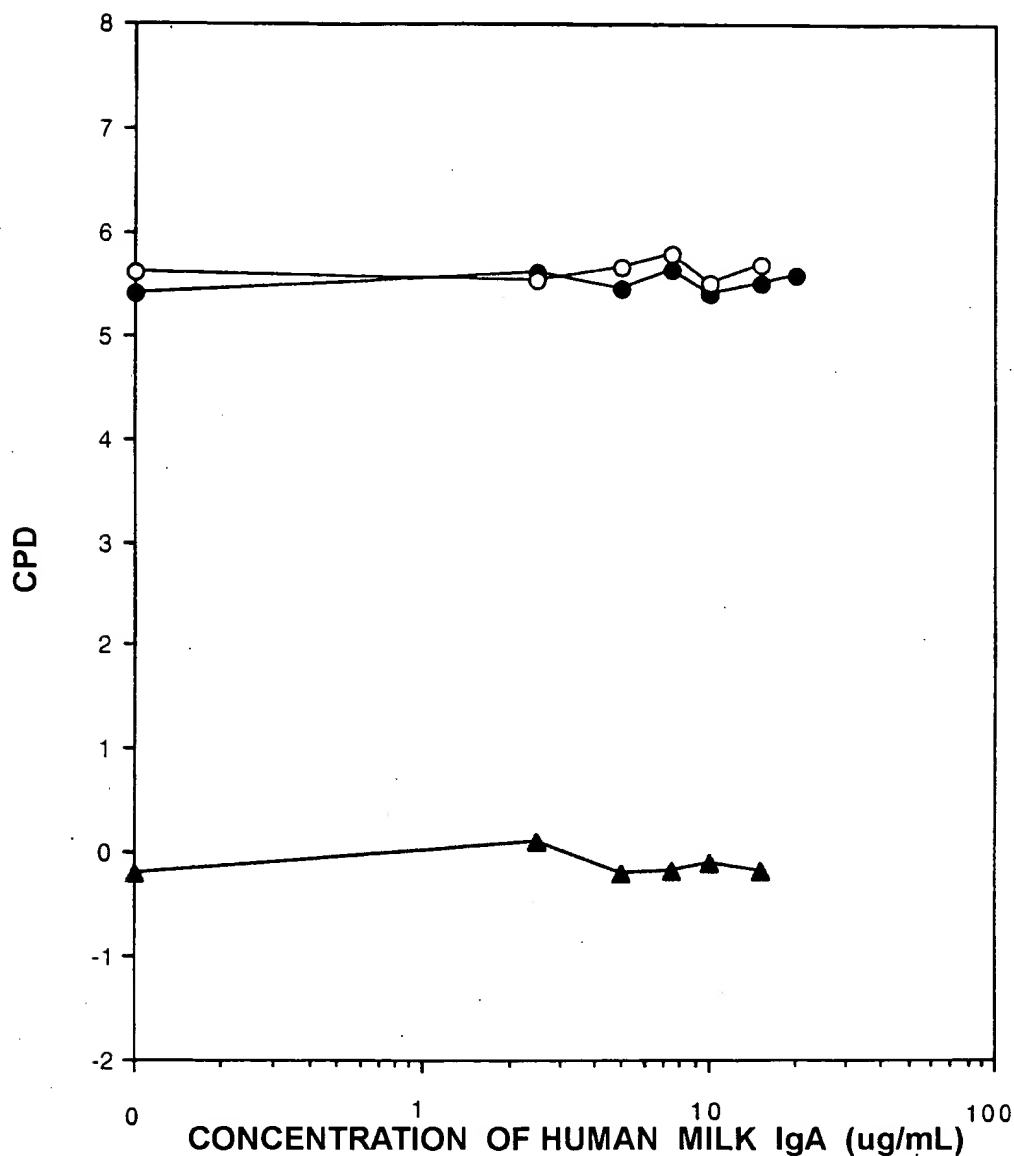


LEGEND:

- = + E₂
- = - E₂
- ▼— = Estrogenic effect

FIGURE 115

EFFECT OF HUMAN MILK SECRETORY IgA ON
GH₃ CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

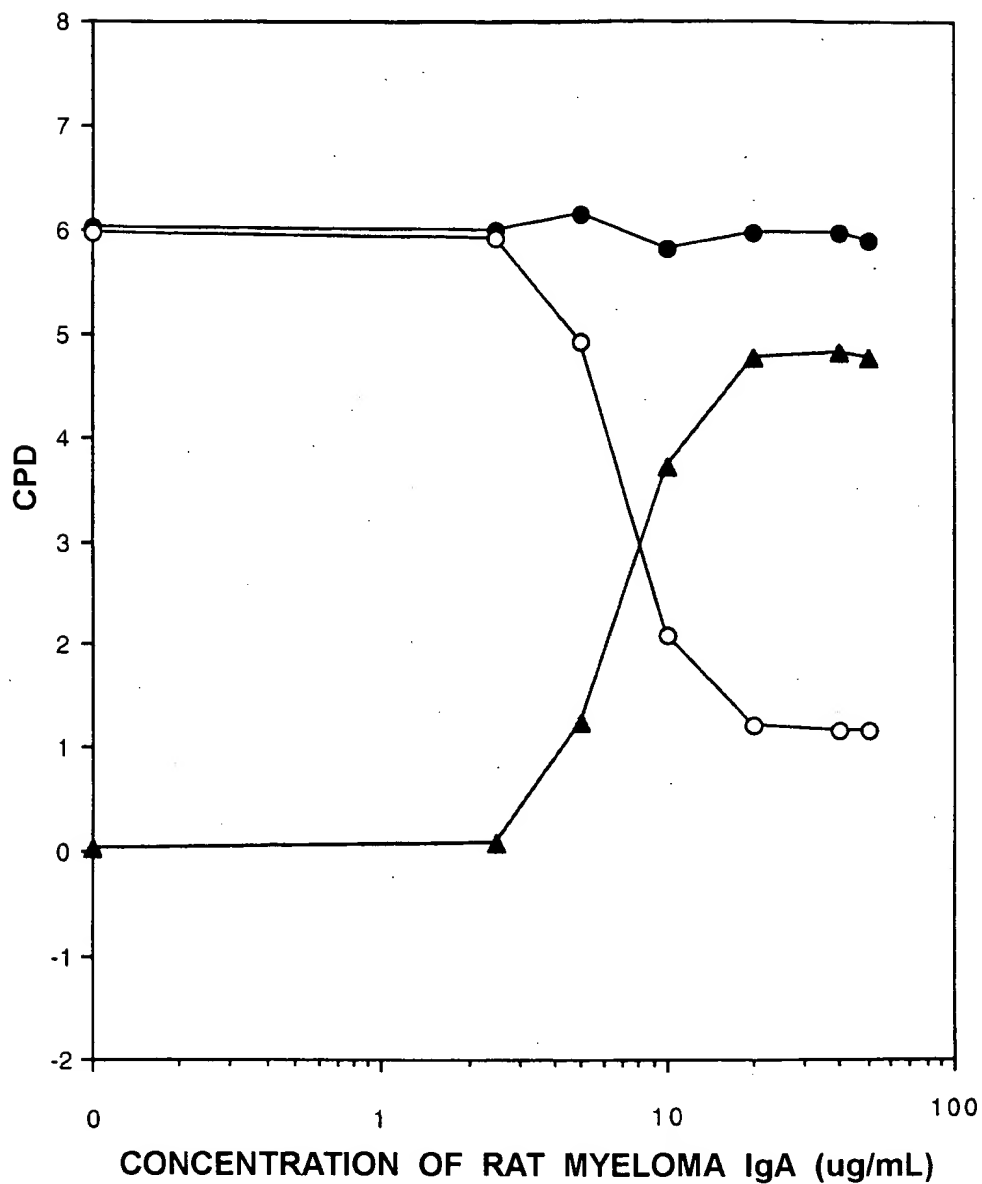
Closed circles = + E₂

Open circles = - E₂

Closed triangles = Estrogenic effect

FIGURE 116

**EFFECT OF RAT MYELOMA IgA ON GH₄
CELL GROWTH IN SERUM-FREE MEDIUM**



LEGEND:

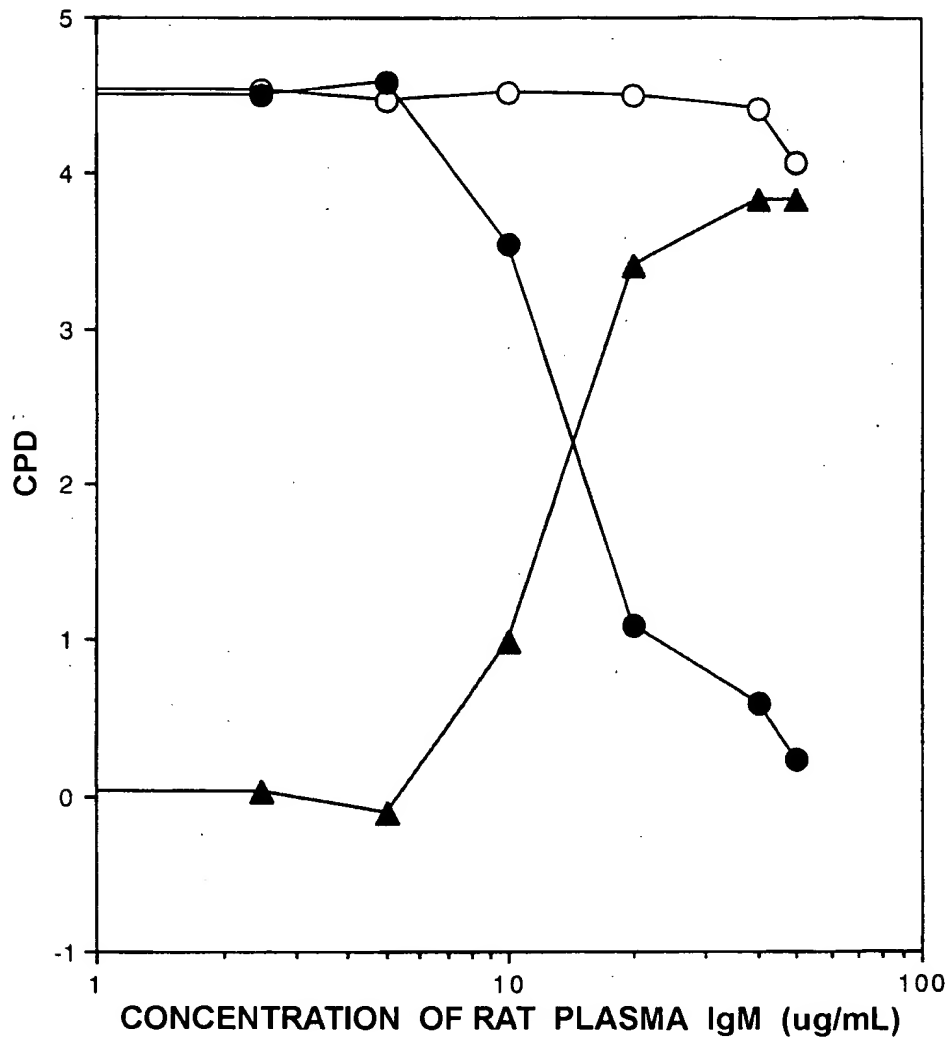
Closed circles = + E₂

Open circles = - E₂

Closed triangles = Estrogenic effect

FIGURE 117

EFFECT OF RAT PLASMA IgM ON GH₄
CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

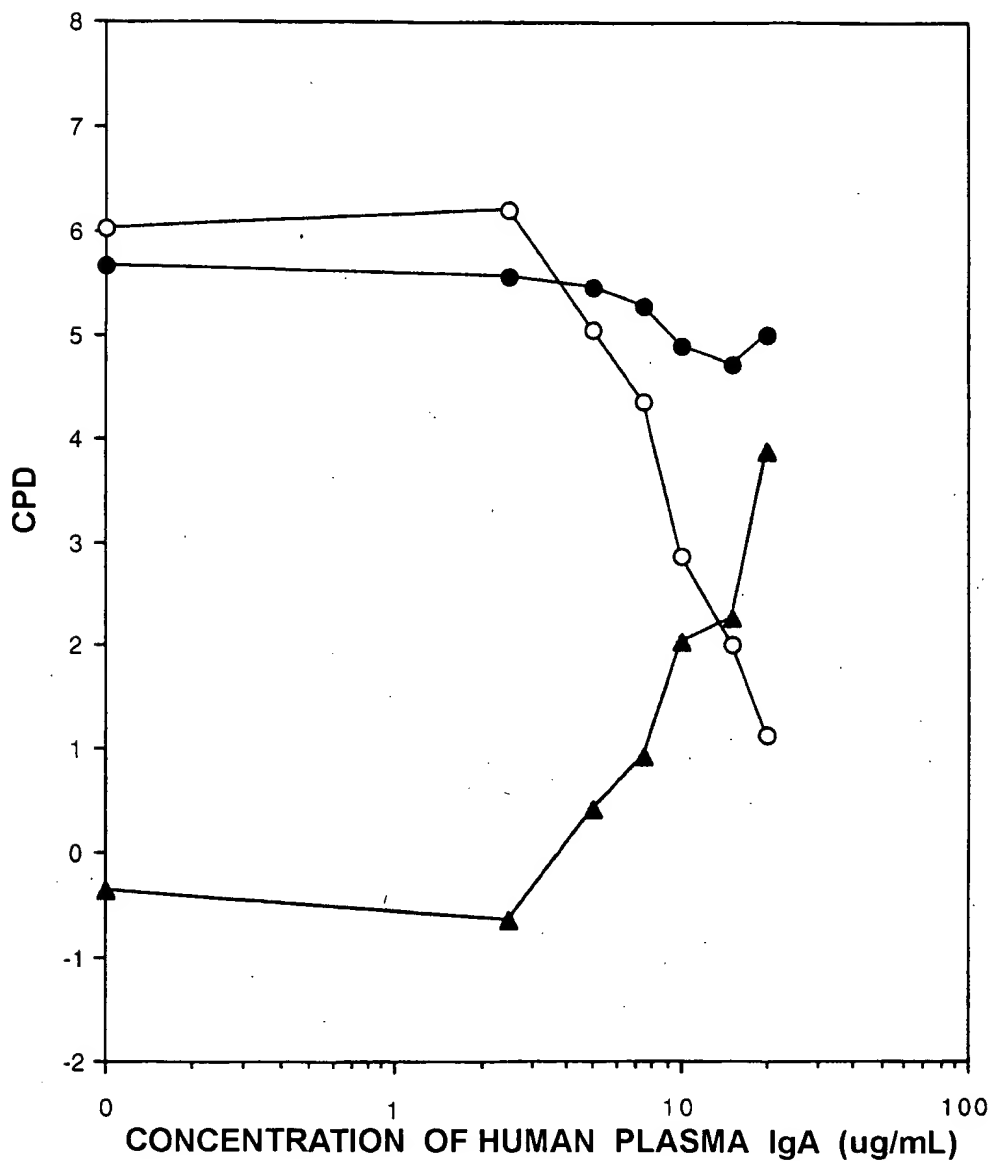
—○— = + E₂

—●— = - E₂

—▲— = Estrogenic effect

FIGURE 118

EFFECT OF HUMAN PLASMA IgA ON GH₄C₁
CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

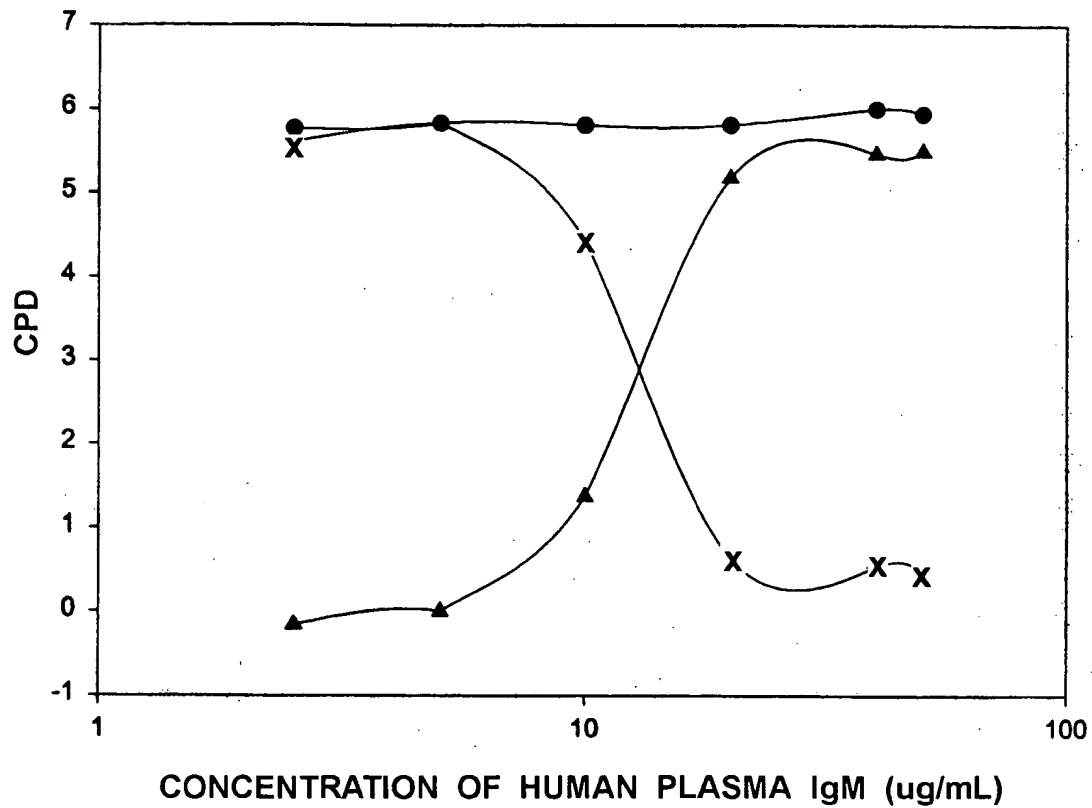
Closed circles = + E₂

Open circles = - E₂

Closed triangles = Estrogenic effect

FIGURE 119

EFFECT OF HUMAN PLASMA IgM ON GH₄C₁
CELL GROWTH IN SERUM-FREE MEDIUM

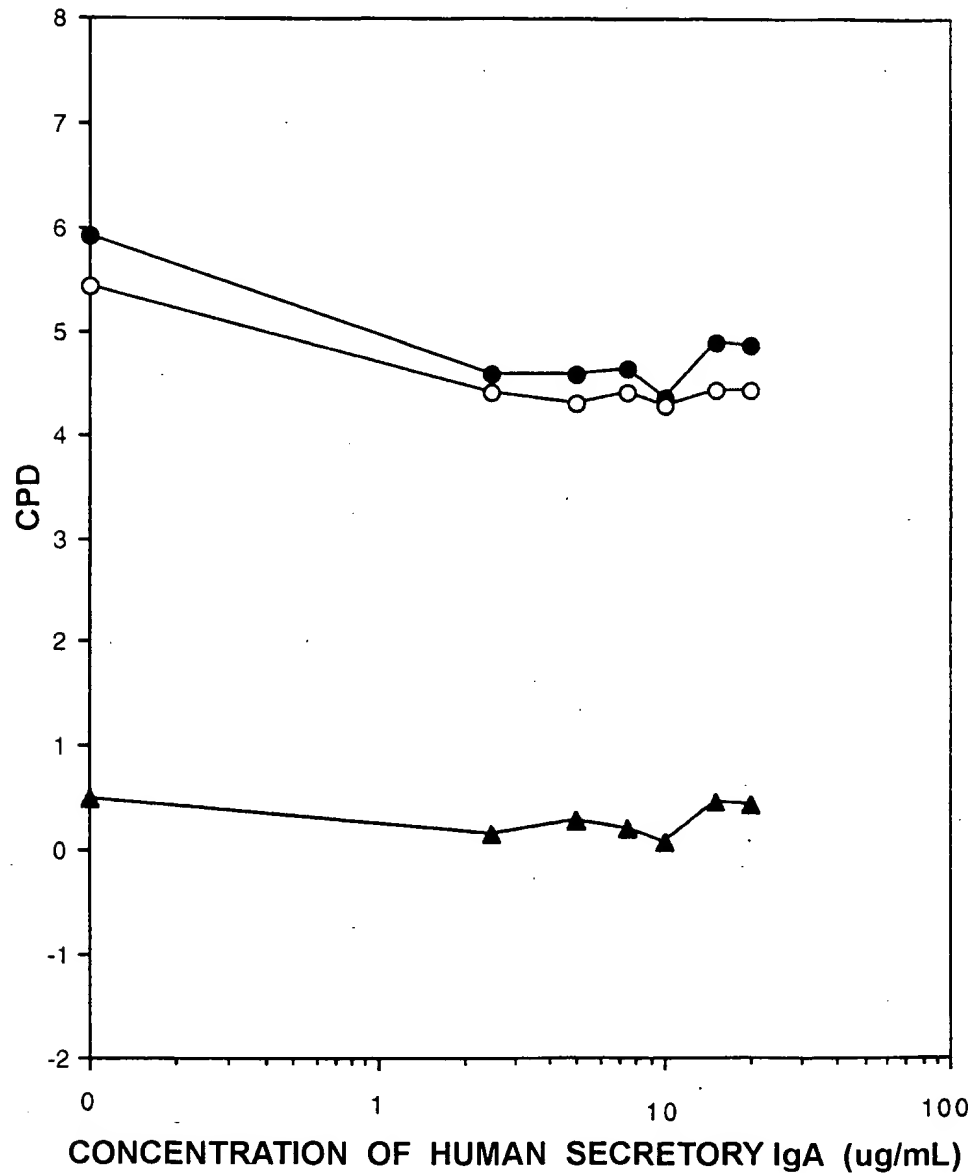


LEGEND:

- = + E₂
- X— = - E₂
- ▲— = Estrogenic effect

FIGURE 120

EFFECT OF HUMAN MILK SECRETORY IgA ON
GH₄C₁ CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

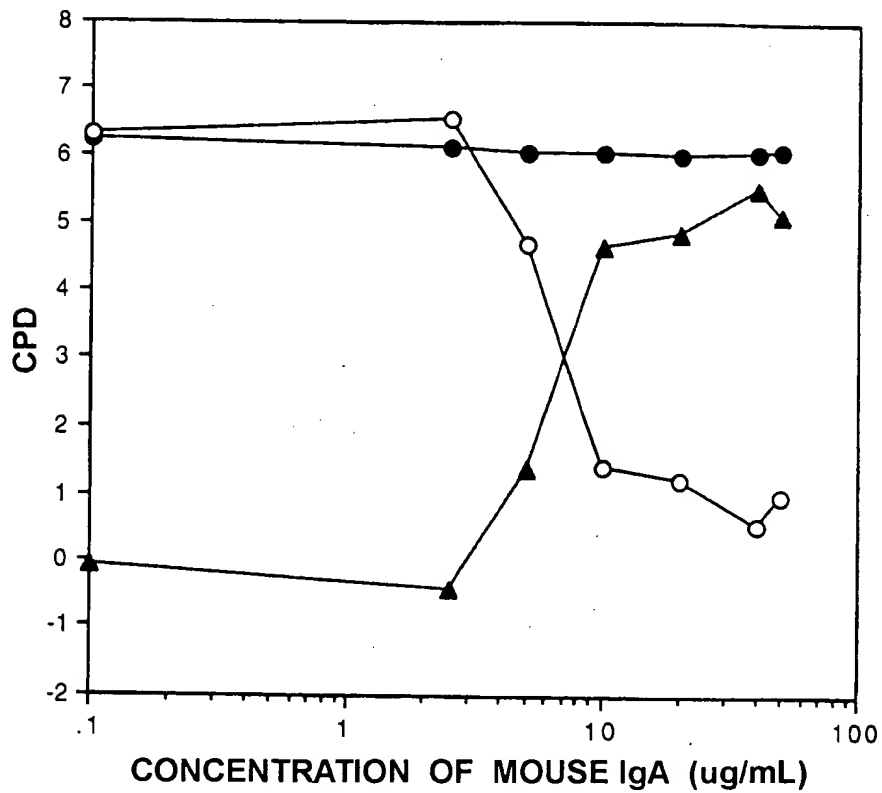
Closed circles = + E₂

Open circles = - E₂

Closed triangles = Estrogenic effect

FIGURE 121

EFFECT OF MOUSE IgA ON H301 CELL
GROWTH IN SERUM-FREE MEDIUM



LEGEND:

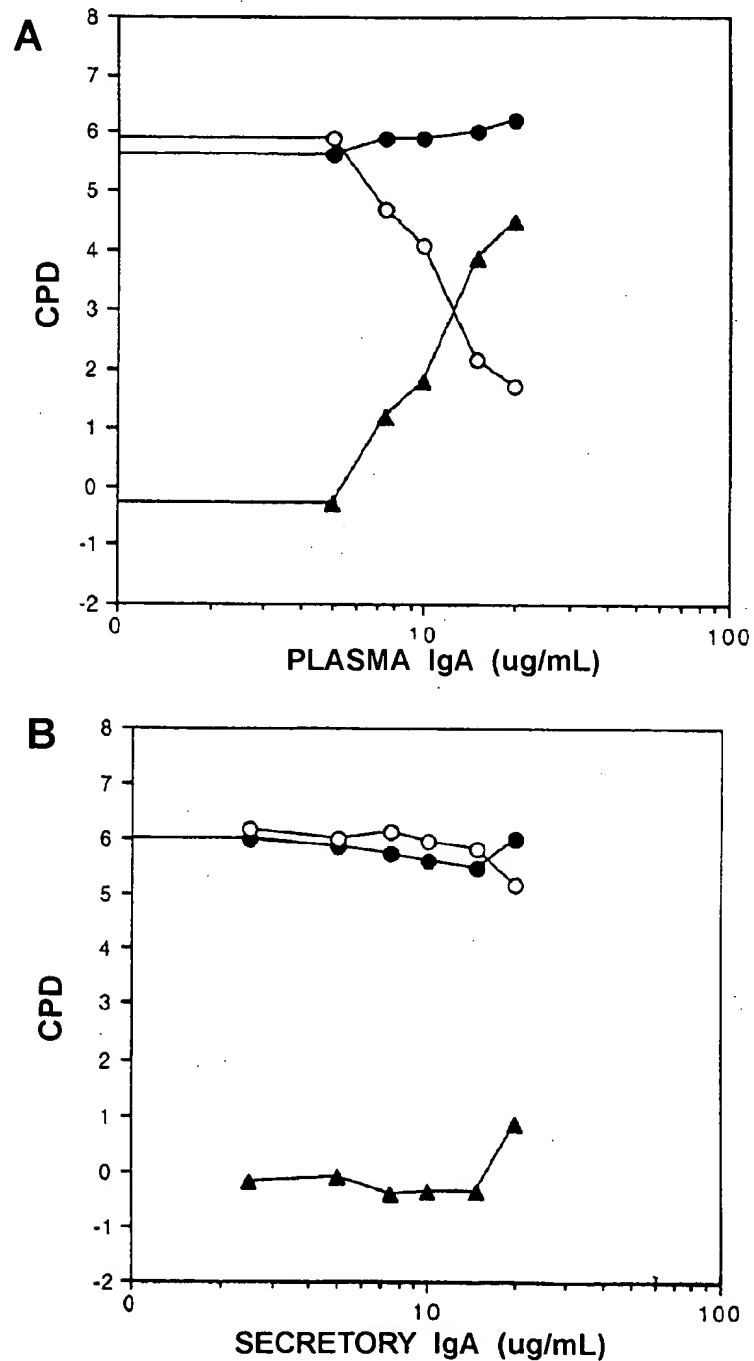
Closed circles = + E₂

Open circles = - E₂

Closed triangles = Estrogenic effect

FIGURE 122

EFFECT OF HUMAN PLASMA IgA (A) AND SECRETORY IgA (B) ON H301CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND: Closed circles = + E₂
 Open circles = - E₂
 Closed triangles = Estrogenic effect

FIGURE 123

EFFECT OF ESTRADIOL ON H301 CELL GROWTH IN
SERUM-FREE MEDIUM AND 40 ug/mL OF HUMAN IgM

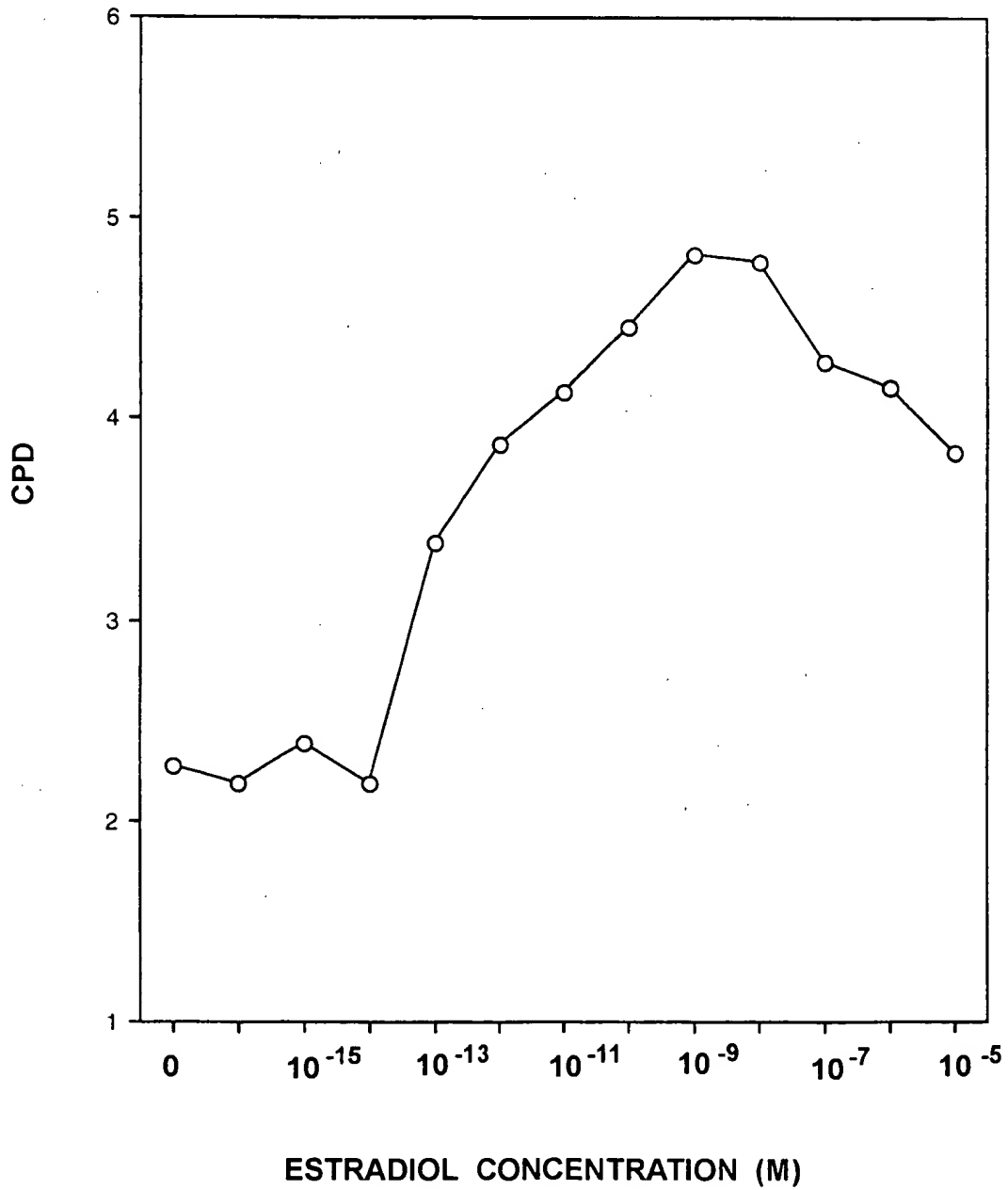
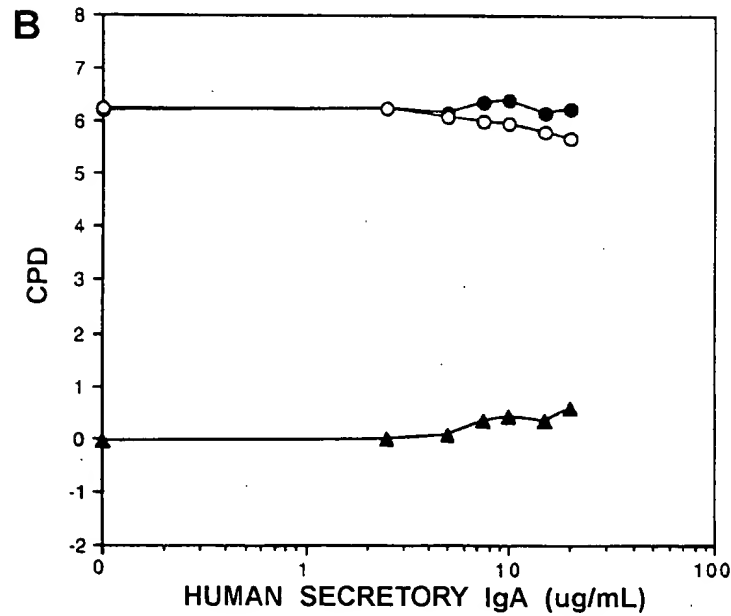
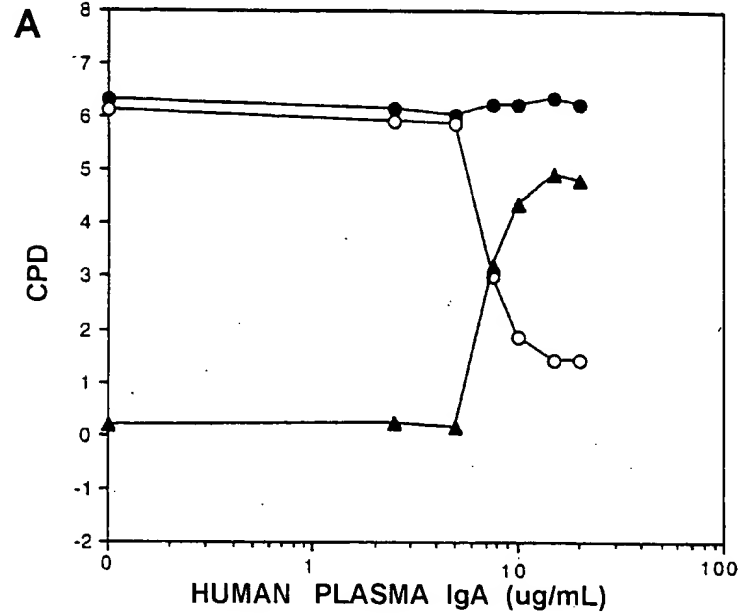


FIGURE 124

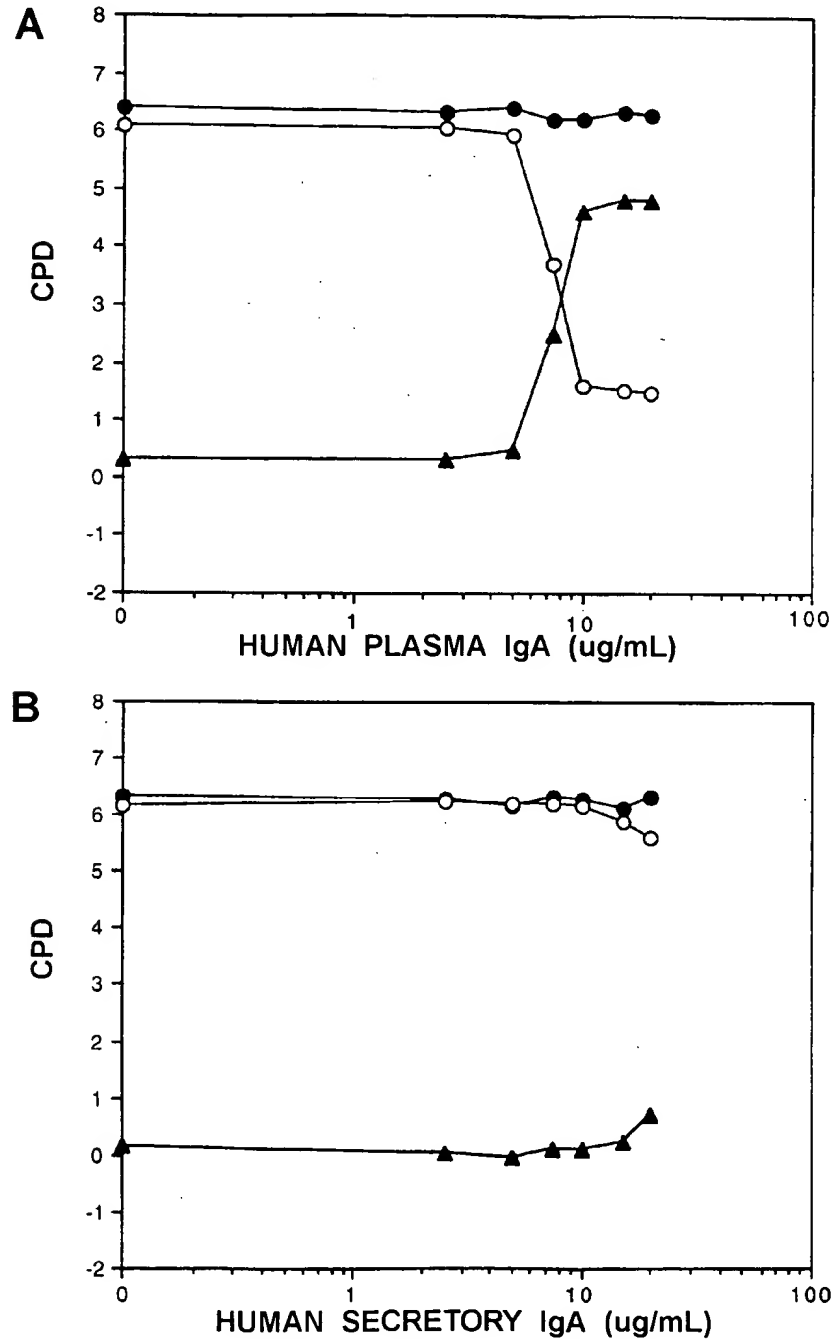
EFFECT OF HUMAN PLASMA IgA (A) AND SECRETORY IgA (B) ON MCF-7A CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND: Closed circles = + E₂
 Open circles = - E₂
 Closed triangles = Estrogenic effect

FIGURE 125

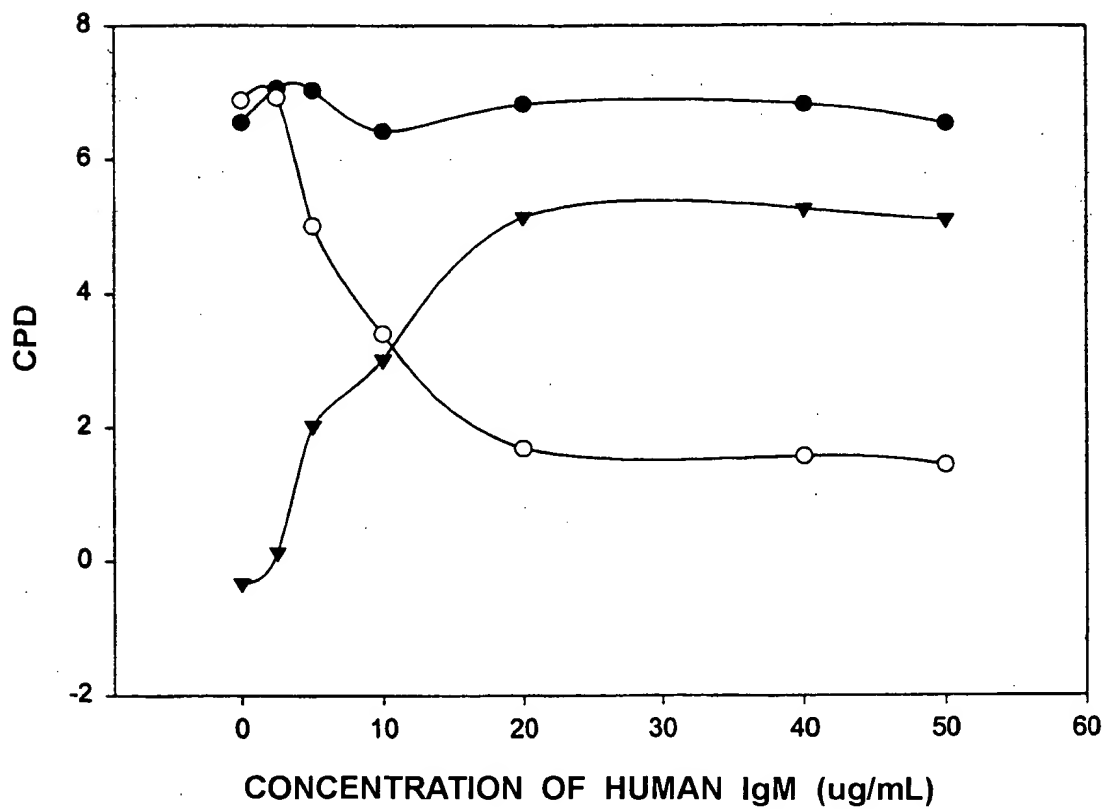
EFFECT OF HUMAN PLASMA IgA (A) AND SECRETORY IgA (B) ON MCF-7K CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND: Closed circles = + E₂
 Open circles = - E₂
 Closed triangles = Estrogenic effect

FIGURE 126

EFFECT OF HUMAN IgM ON MCF-7A CELL
GROWTH IN SERUM-FREE MEDIUM

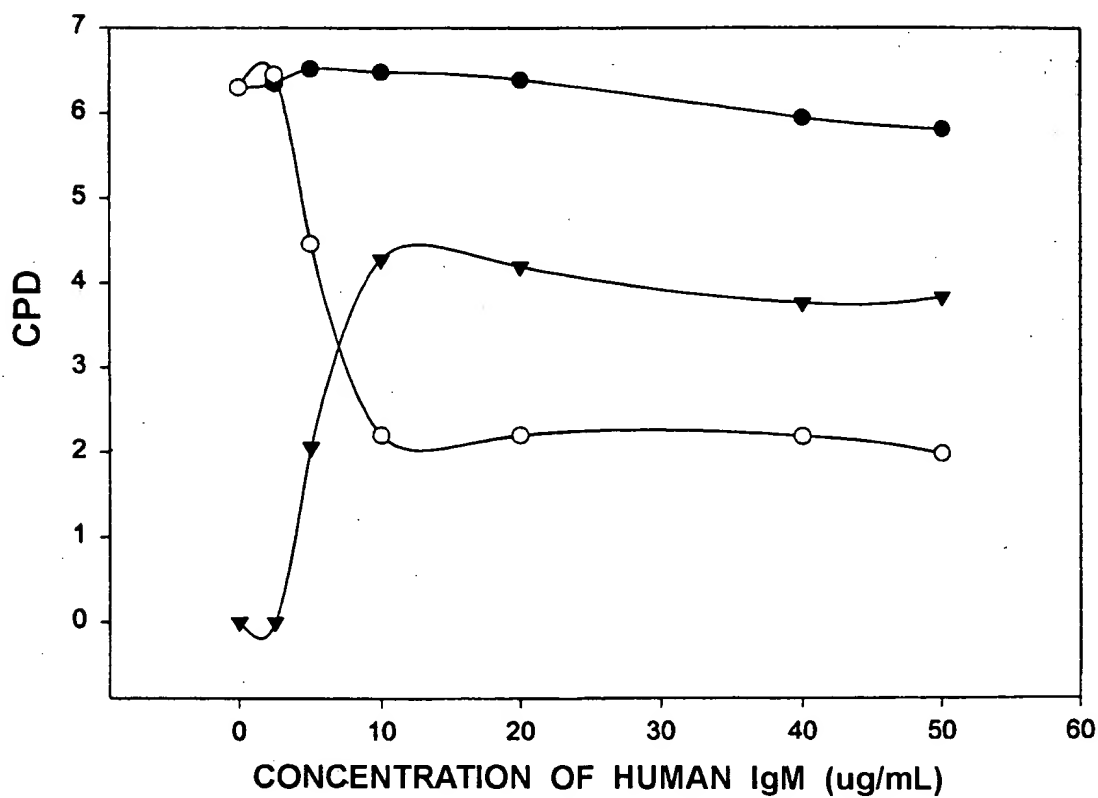


LEGEND:

- = + E_2
- = - E_2
- ▼ = Estrogenic effect

FIGURE 127

EFFECT OF HUMAN IgM ON MCF-7K
CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

- = + E₂
- = - E₂
- ▼ = Estrogenic effect

FIGURE 128

EFFECT OF ESTRADIOL ON MCF-7K CELL GROWTH
IN SERUM-FREE MEDIUM WITH 40 ug/mL HUMAN IgM

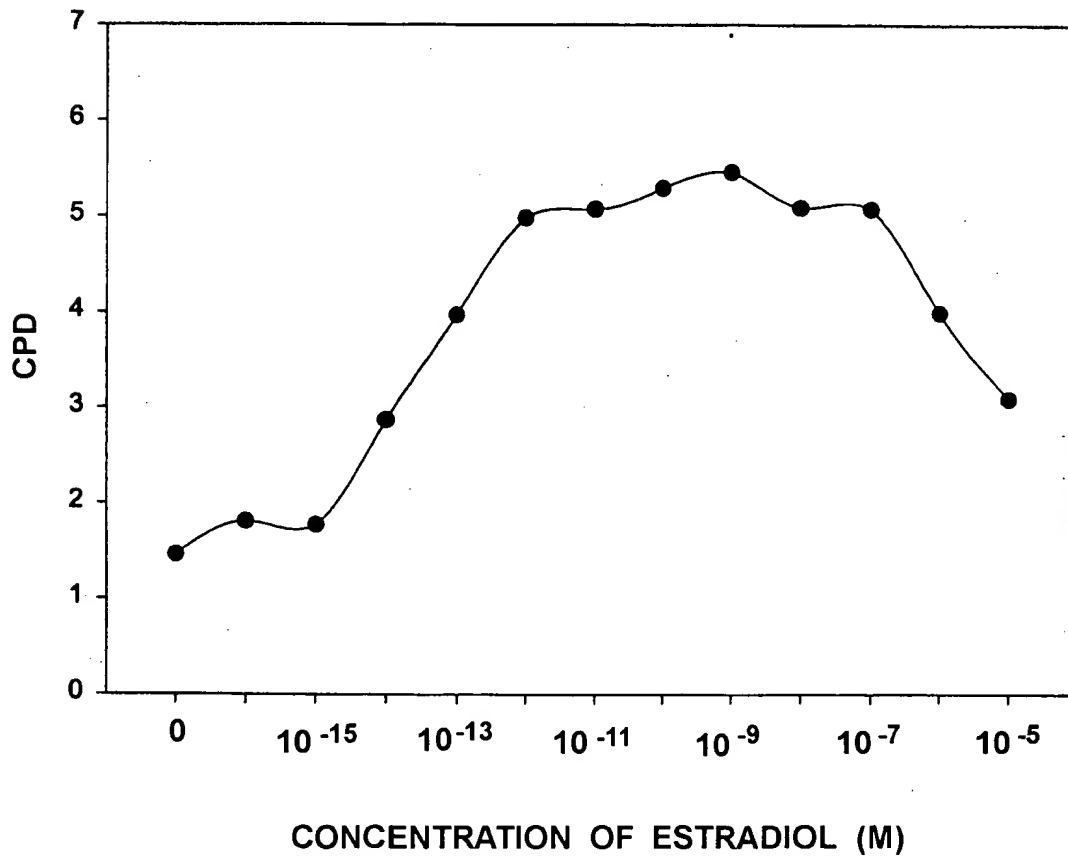
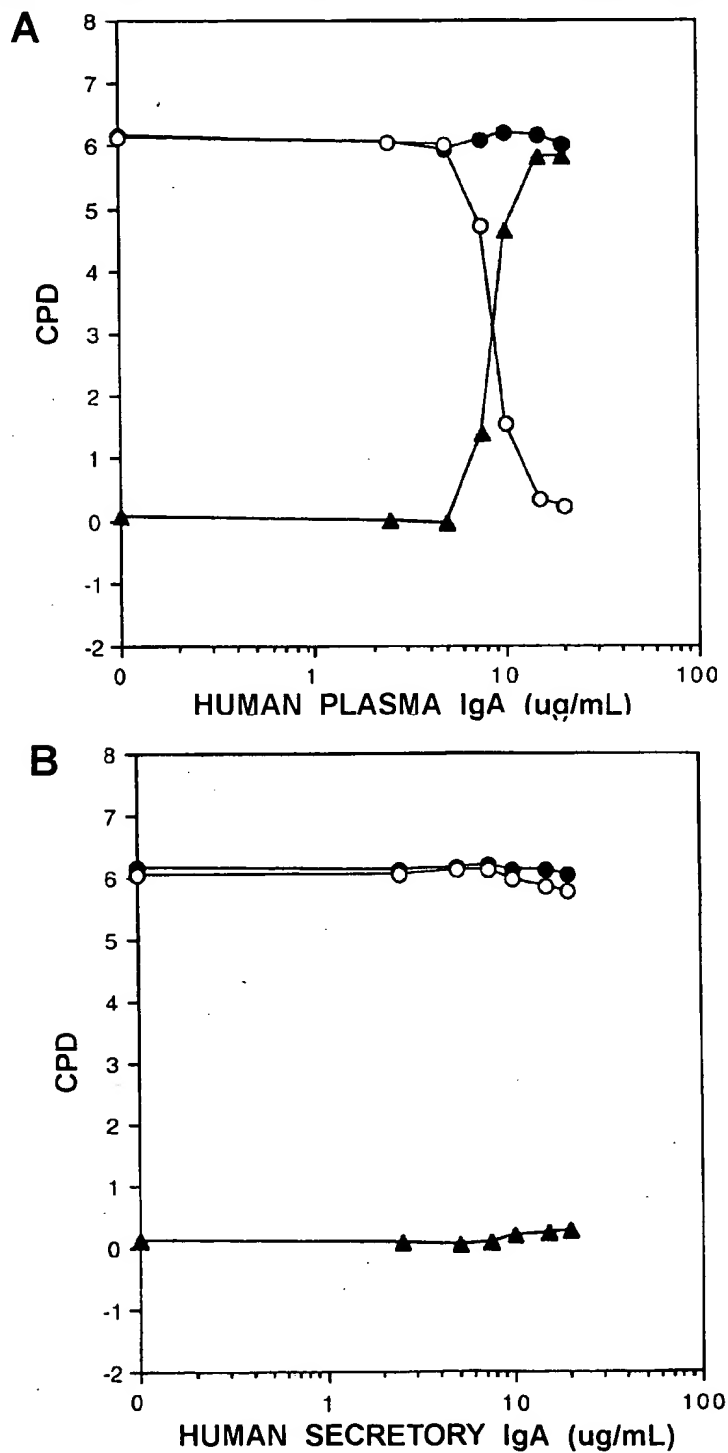


FIGURE 129

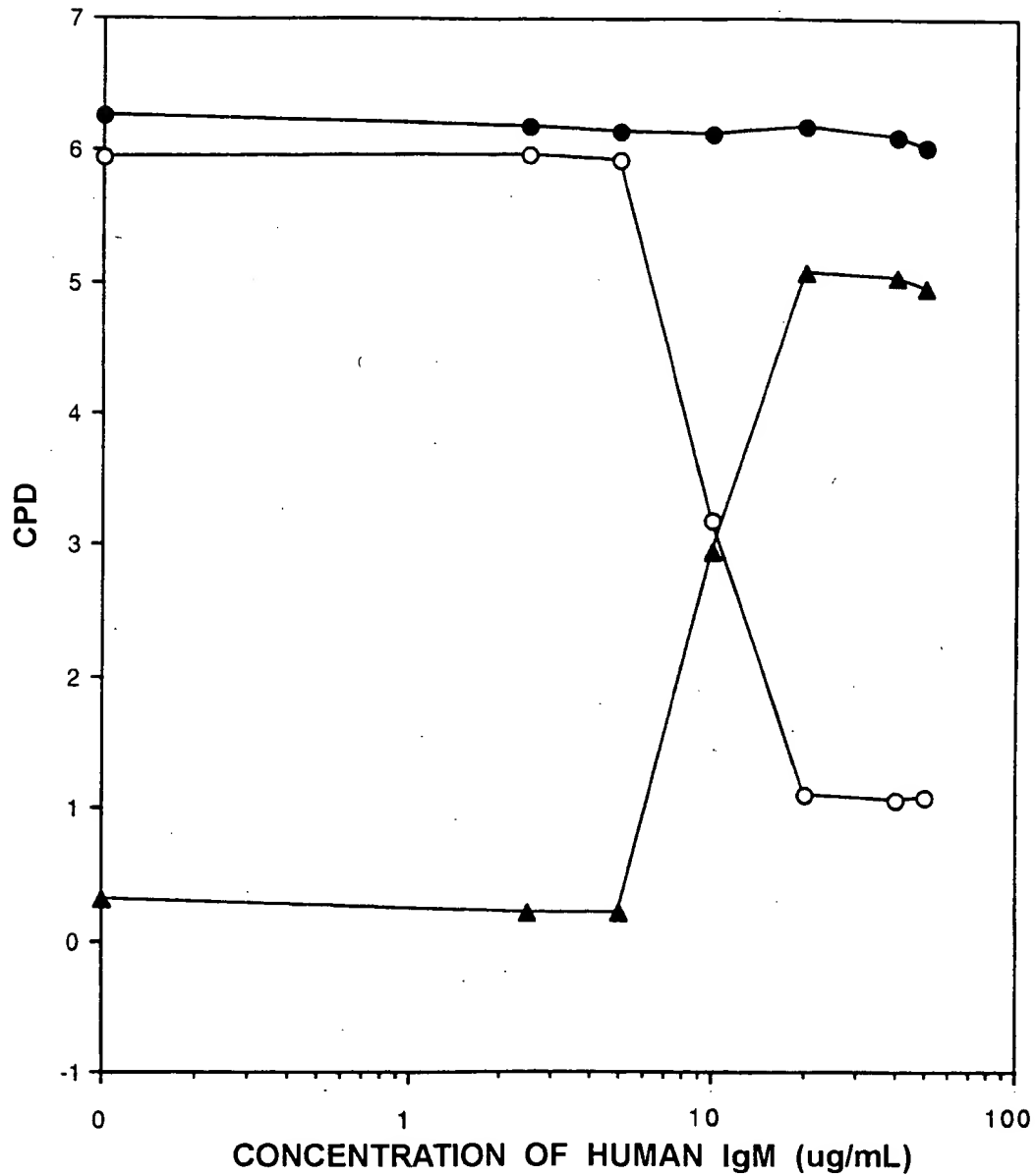
EFFECT OF HUMAN PLASMA IgA (A) AND SECRETORY IgA (B) ON T47D CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND: Closed circles = + E₂
 Open circles = - E₂
 Closed triangles = Estrogenic effect

FIGURE 130

**EFFECT OF HUMAN IgM ON T47D CELL
GROWTH IN SERUM-FREE MEDIUM**



LEGEND: Closed circles = + E₂
 Open circles = - E₂
 Closed triangles = Estrogenic effect

FIGURE 131

EFFECT OF ESTRADIOL ON T47D CELL GROWTH IN
SERUM-FREE MEDIUM WITH 40 ug/mL HUMAN IgM

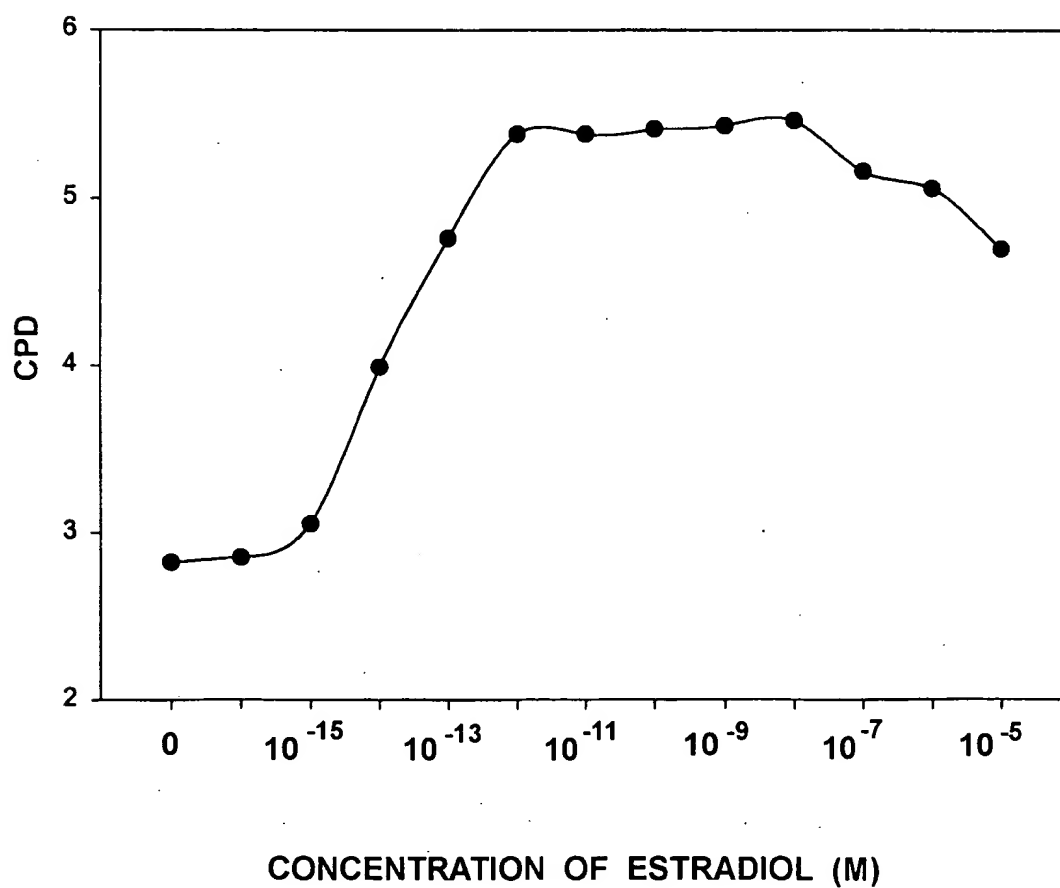
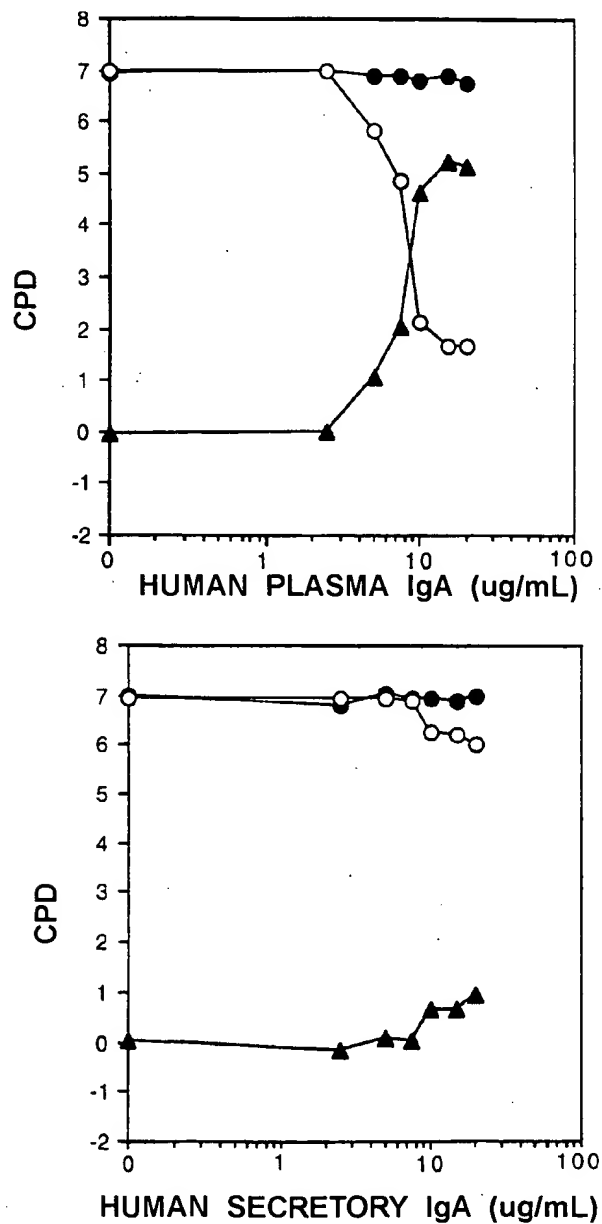


FIGURE 132

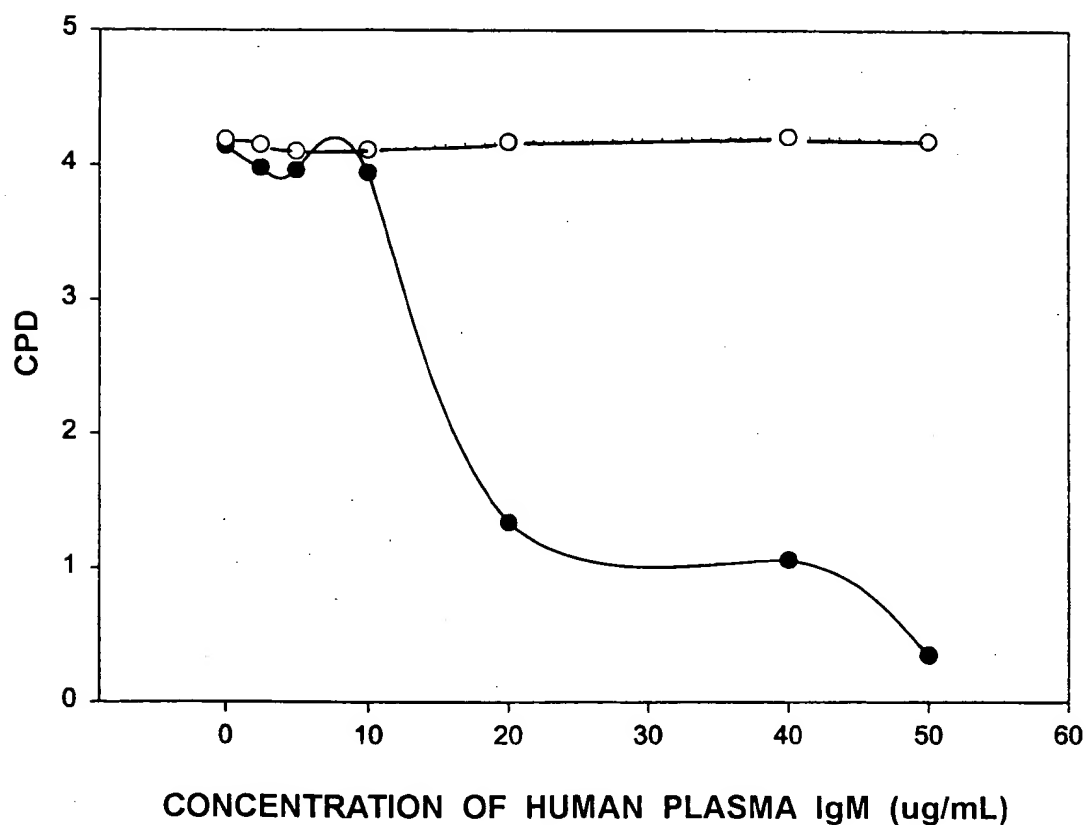
EFFECT OF HUMAN PLASMA IgA (A) AND SECRETORY
IgA (B) ON ZR-75-1 CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND: Closed circles = + E₂
Open circles = - E₂
Closed triangles = Estrogenic effect

FIGURE 133

EFFECT OF HUMAN PLASMA IgM ON
ZR-75-1 CELL GROWTH IN SERUM-FREE MEDIUM



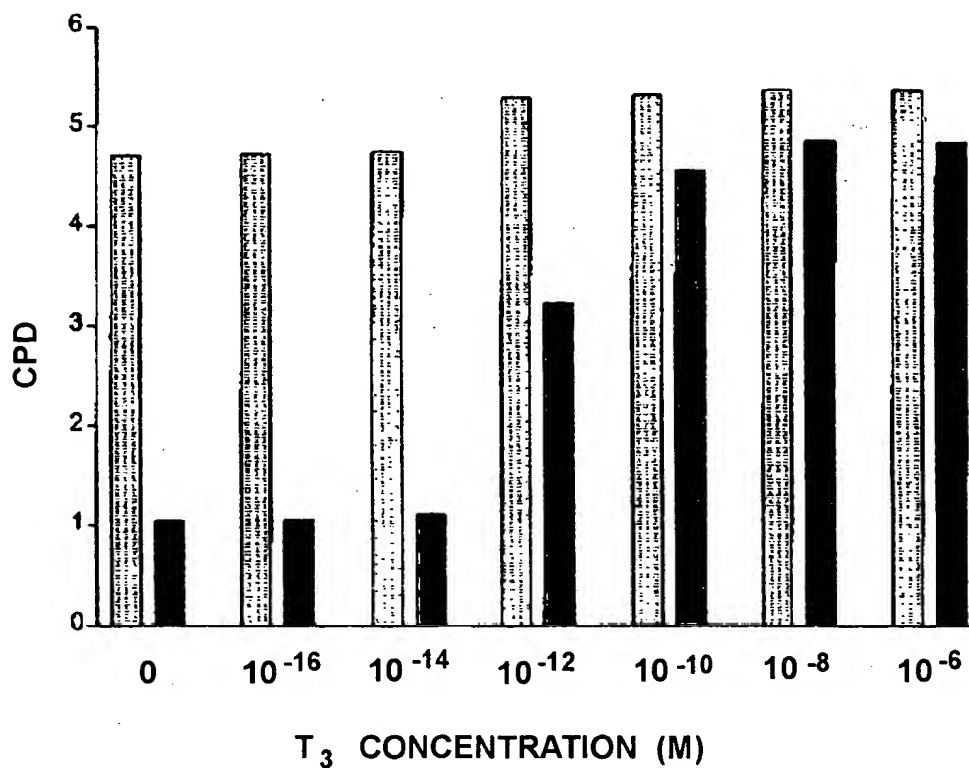
LEGEND:

—●— = - E₂


-○- = + E₂

FIGURE 134

EFFECT OF HUMAN IgM ON HT-29 CELL GROWTH IN
THE PRESENCE OF INCREASING CONCENTRATIONS OF T_3



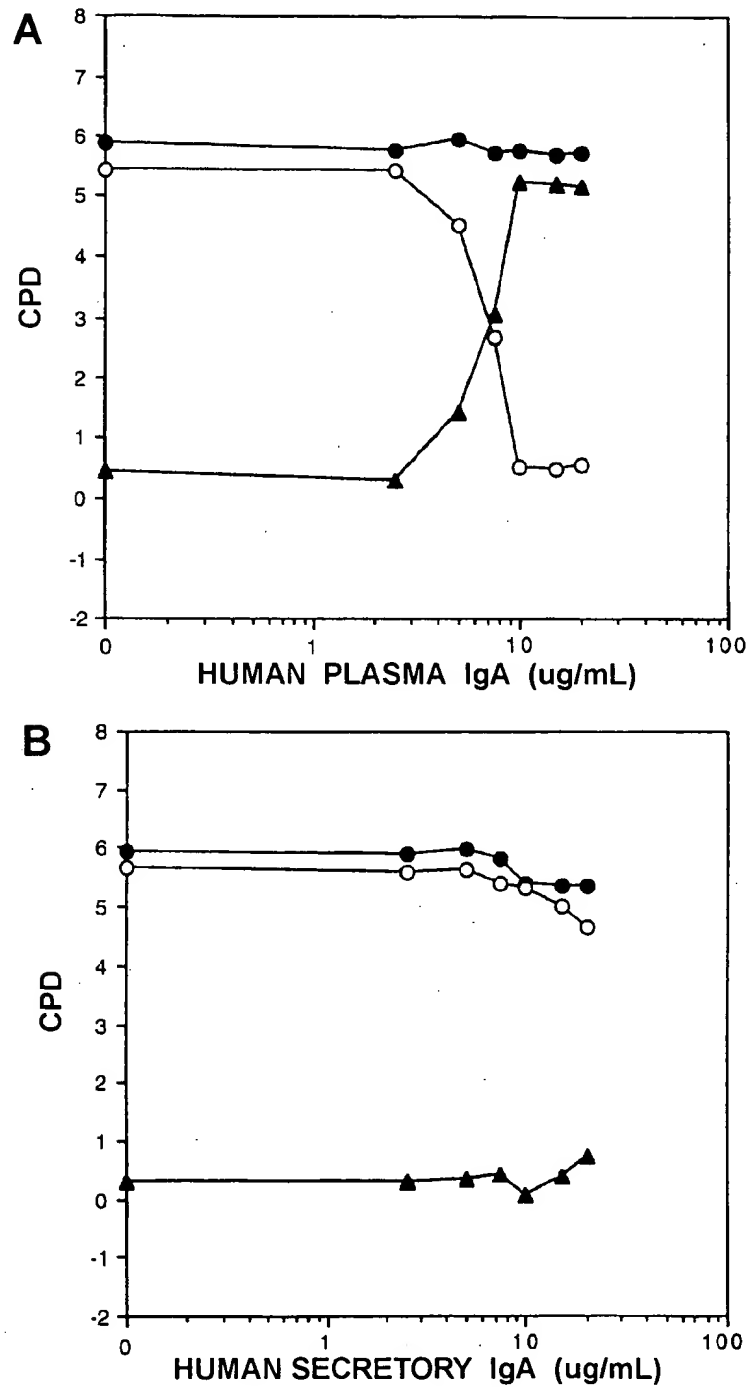
LEGEND:

 = T_3 Titration

 = T_3 Titration + 40 ug/mL IgM

FIGURE 135

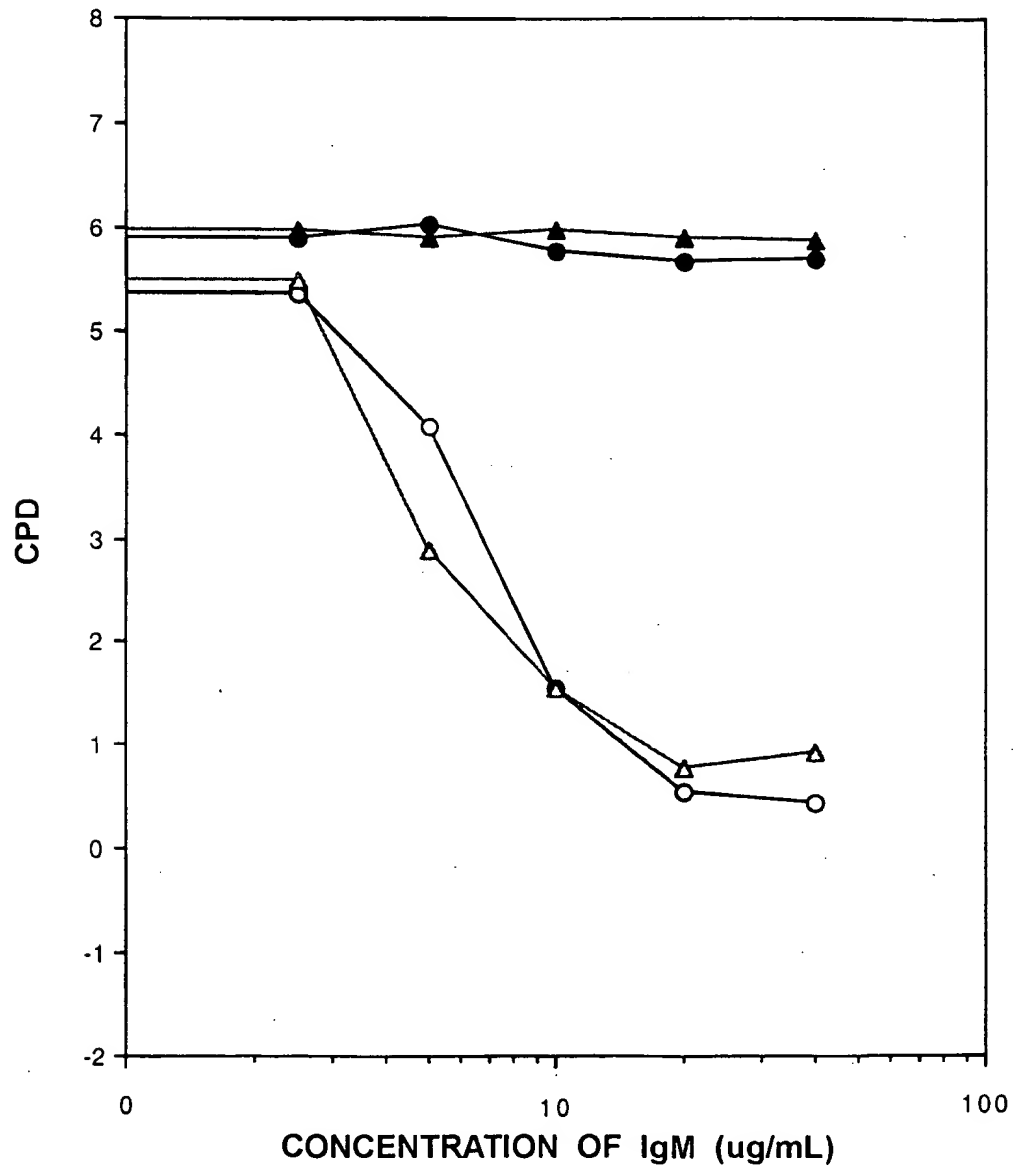
EFFECT OF HUMAN PLASMA IgA (A) AND SECRETORY IgA (B) ON LNCaP CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND: Closed circles = + E₂
Open circles = - E₂
Closed triangles = Estrogenic effect

FIGURE 136

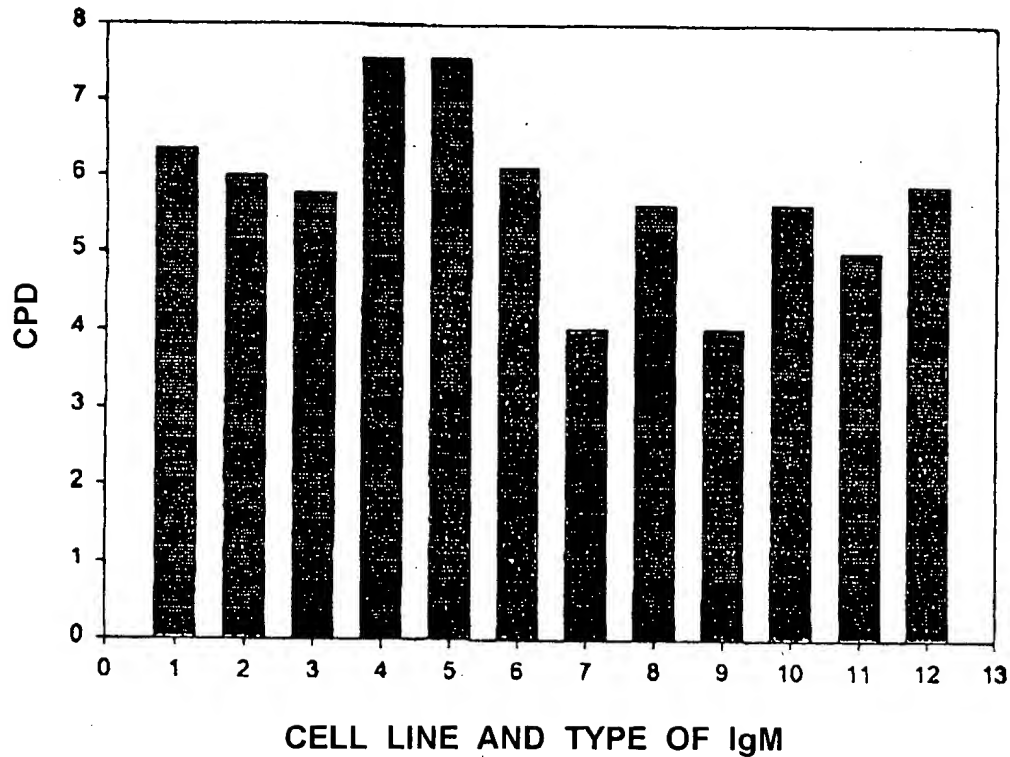
EFFECTS OF HUMAN PLASMA IgM VS IgM DERIVED
FROM MYELOMA CELLS ON LNCaP CELL GROWTH
IN SERUM-FREE MEDIUM WITH AND WITHOUT DHT



LEGEND: —●— = DHT + Myeloma IgM
—○— = Myeloma IgM only
—▲— = DHT + Plasma IgM
—△— = Plasma IgM only

FIGURE 137

**ESTROGENIC EFFECT OF 50 ug/mL OF VARIOUS
IgM'S ON SEVERAL DIFFERENT CELL LINES**

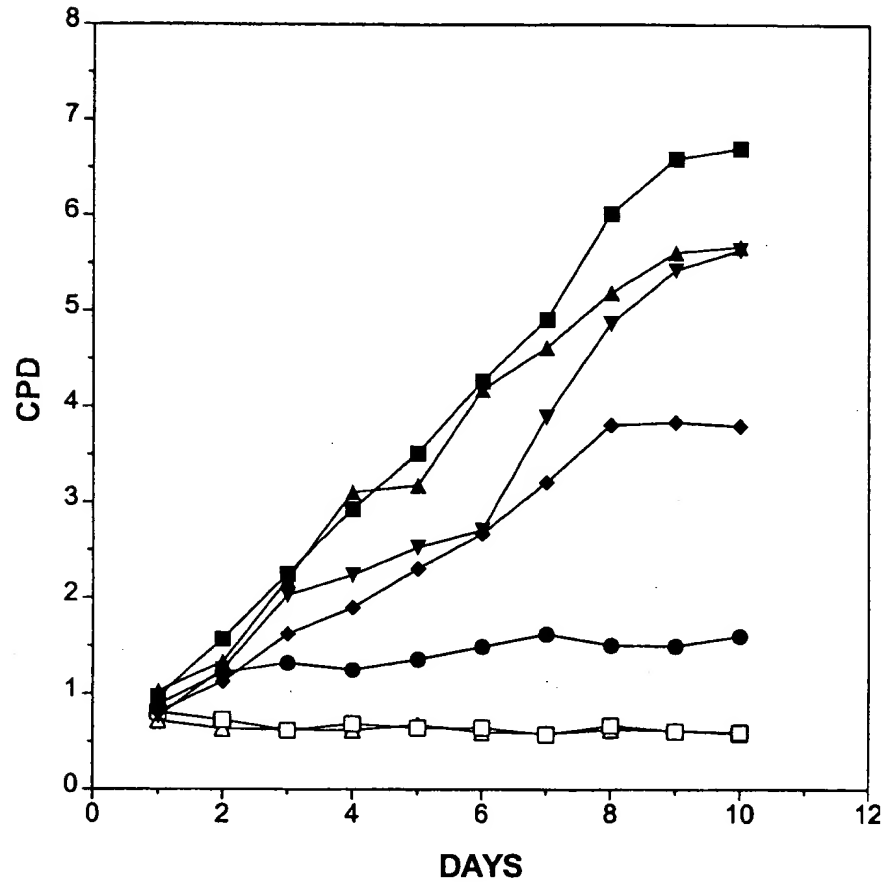


LEGEND:

1. Human IgM on MTW9/PL2 Cells = 6.36 cpd
2. Mouse IgM on MTW9/PL2 Cells = 6.00 cpd
3. Rat IgM on MTW9/PL2 Cells = 5.77 cpd
4. Human IgM on H301 Cells = 7.57 cpd
5. Mouse IgM on H301 Cells = 7.56 cpd
6. Rat IgM on H301 Cells = 6.11 cpd
7. Human IgM on GH1 Cells = 4.12 cpd
8. Rat IgM on GH1 Cells = 5.83 cpd
9. Human IgM on GH3 Cells = 4.09 cpd
10. Human IgM on GH4 Cells = 5.41 cpd
11. Human IgM on MCF-7A Cells = 5.01 cpd
12. Human IgM on MCF-7K Cells = 5.89 cpd

FIGURE 138

EFFECT OF TAMOXIFEN ON T47D CELL GROWTH
IN DDM-2MF DEFINED MEDIUM

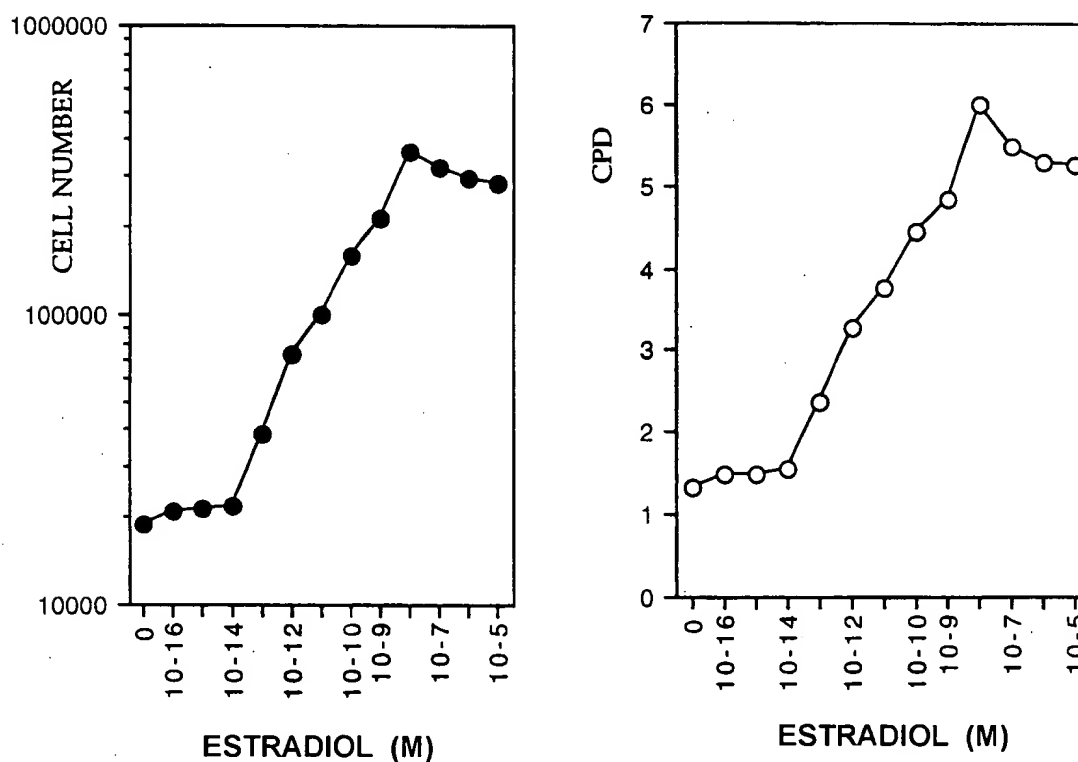


LEGEND:

- SFM + E₂
- ▲ SFM - E₂
- ▼ SFM + 10⁻⁹ M TAM
- ◆ SFM + 10⁻⁸ M TAM
- SFM + 10⁻⁷ M TAM
- SFM + 10⁻⁶ M TAM
- △ SFM + 10⁻⁵ M TAM

FIGURE 139

**EFFECT OF INCREASING ESTRADIOL CONCENTRATIONS
ON T47D CELL GROWTH IN SERUM-FREE AND
PHENOL-RED FREE MEDIUM WITH 10^{-7} TAMOXIFEN**

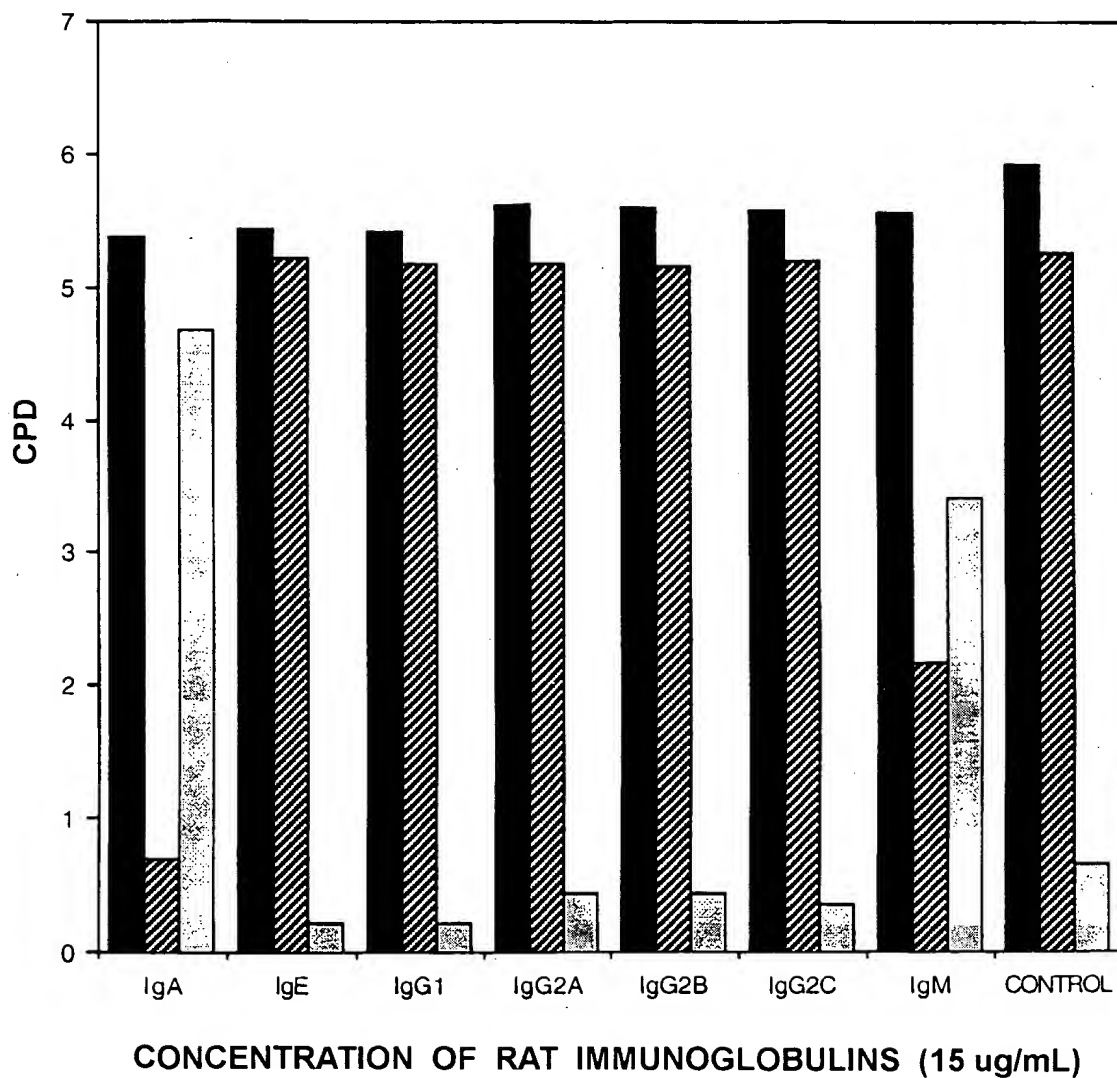


NOTE:

DATA ARE EXPRESSED AS BOTH CELL NUMBER AND CPD

FIGURE 140

EFFECT OF RAT IMMUNOGLOBULINS ON MTW9/PL2
CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

■ = + E₂

▨ = - E₂

□ = Estrogenic effect

CONTROL IS SERUM-FREE MEDIUM ALONE ± E₂

FIGURE 141

**ESTROGENIC EFFECT GENERATED BY IMMUNOGLOBULINS
WITH T47D CELLS IN SERUM-FREE MEDIUM**

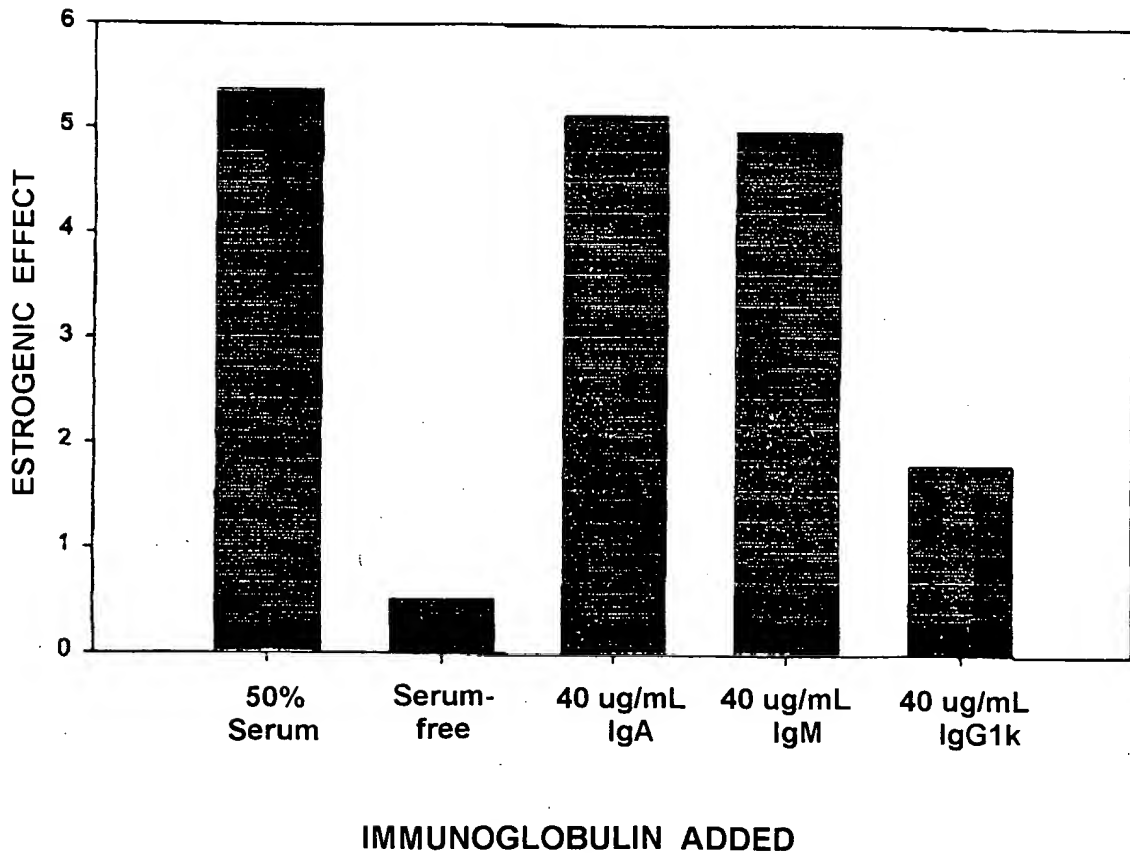
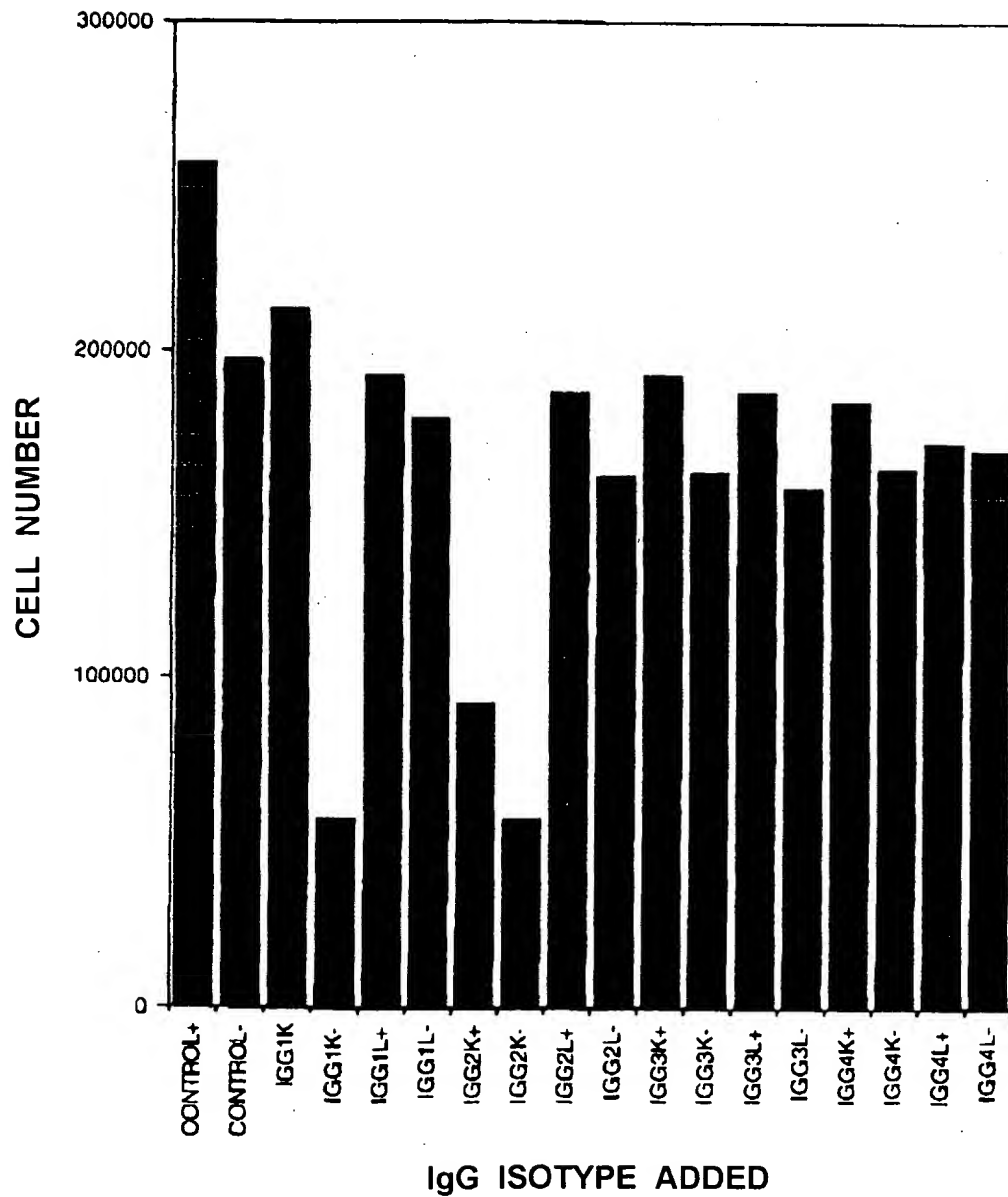


FIGURE 142

**EFFECT OF IgG ISOTYPES (40 ug/mL) ON LNCaP
CELL GROWTH IN SERUM-FREE MEDIUM**

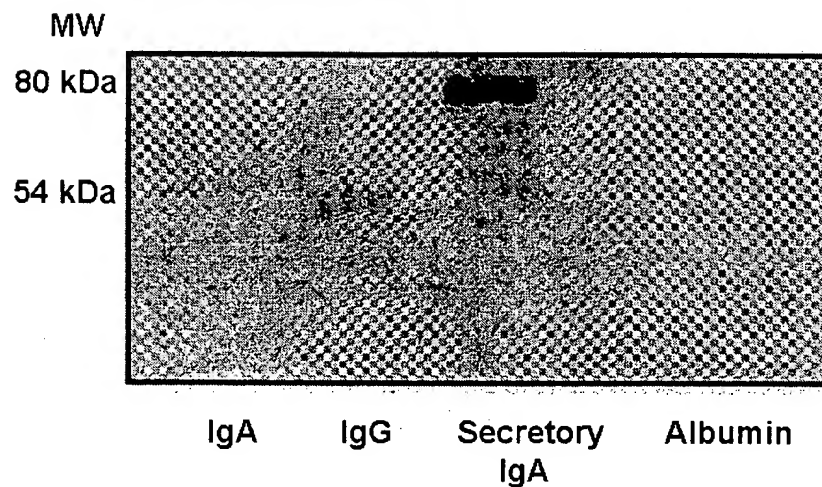


LEGEND: + = DHT Added
- = No DHT Added

FIGURE 143

**DETECTION OF SECRETORY COMPONENT
IN SECRETORY IgA WITH ANTI-SC ANTIBODY**

BEST AVAILABLE COPY



20 ug/mL APPLIED IN ALL LINES

IgA = Human Plasma

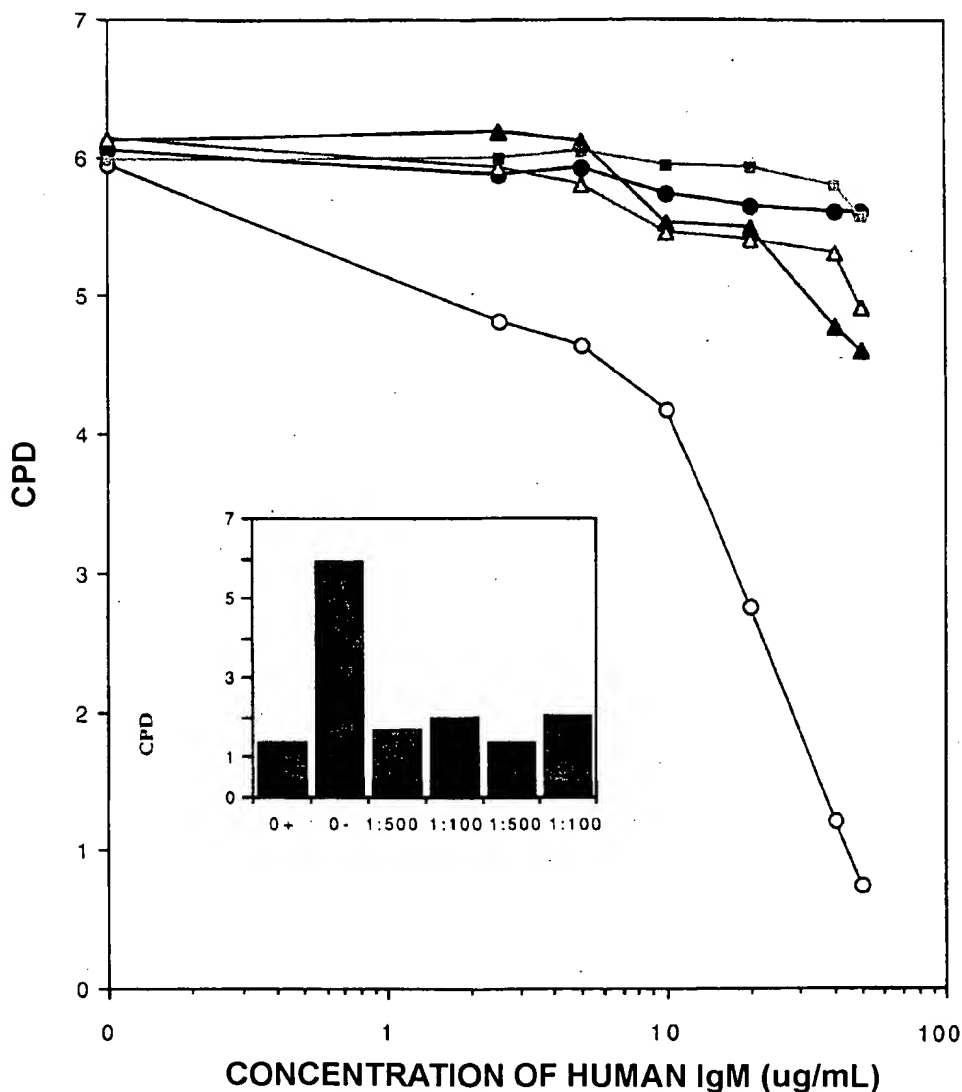
IgG = Human Plasma

Secretory IgA = IgA from Milk

Albumin = Human

FIGURE 144

**HUMAN IgM TITRATION ON T47D CELLS GROWN IN SERUM-FREE
 MEDIUM WITH DIFFERENT DILUTIONS OF ANTI-SC ANTIBODY**

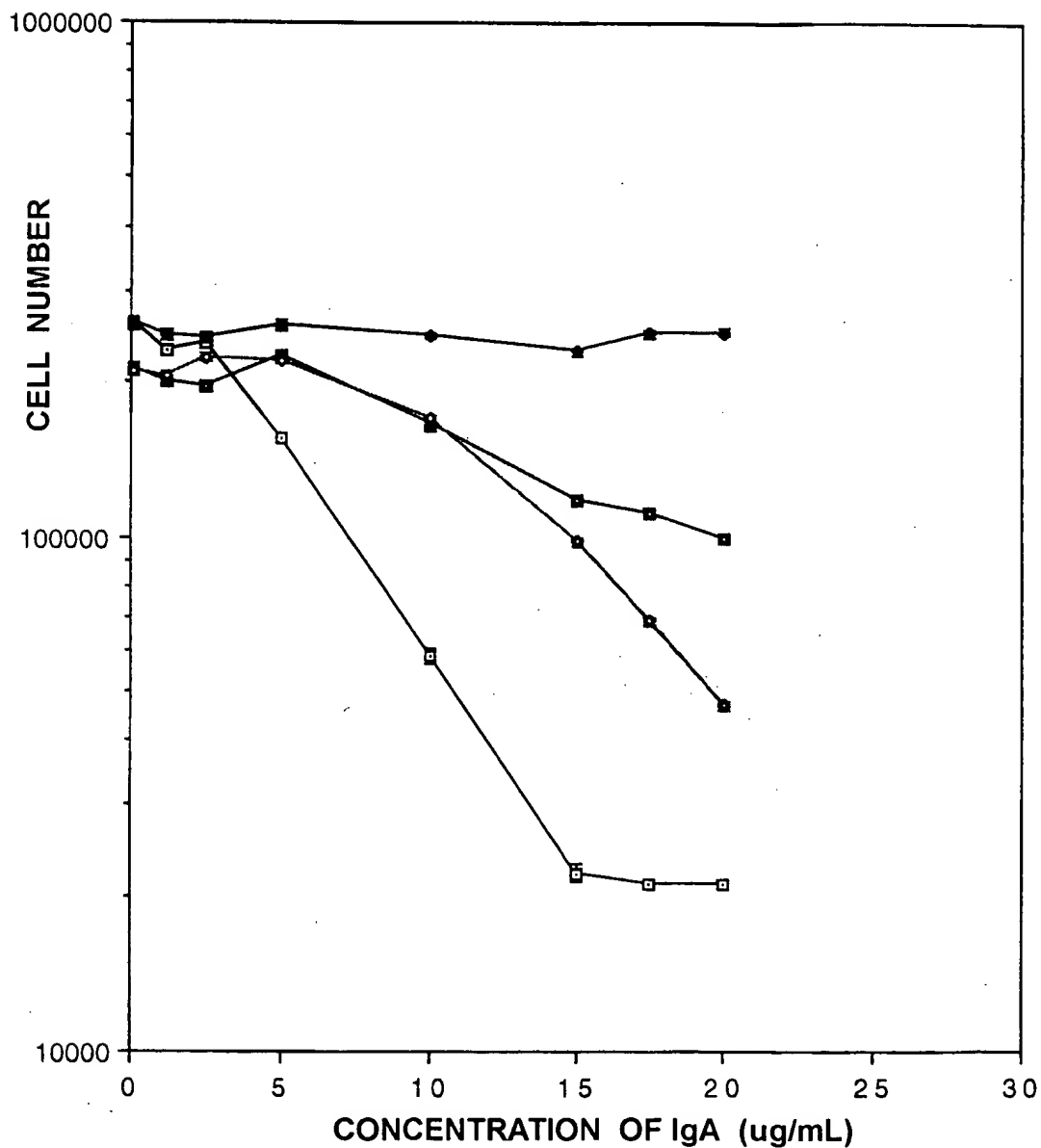


LEGEND: ● = + E₂
 ○ = - E₂
 ▲ = 1:5000 Dilution of Anti-SC Antibody
 △ = 1:1000 Dilution of Anti-SC Antibody
 ■ = 1:500 Dilution of Anti-SC Antibody

**INSERT: EFFECT OF RABBIT SERUM ON T47D CELLS
 INCUBATED WITH 40 ug/mL HUMAN IgM**

FIGURE 145

**EFFECT OF IgA ON LNCaP GROWTH IN THE
PRESENCE OF ANTI-SECRETORY COMPONENT
ANTIBODY AT DIFFERENT DILUTIONS**



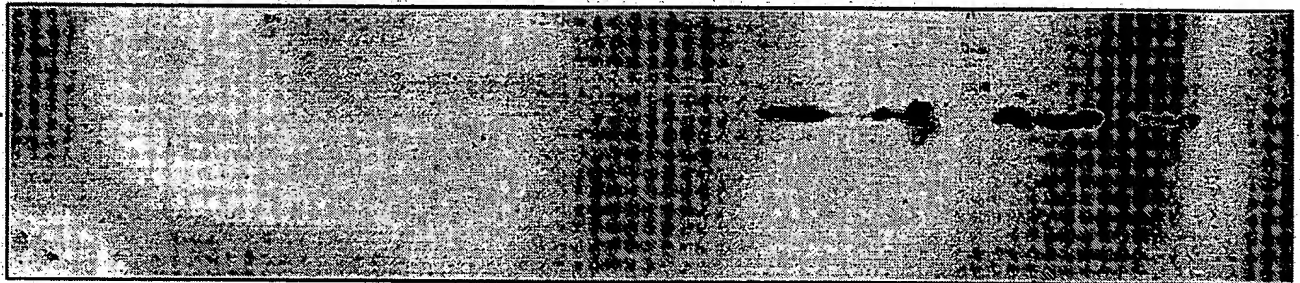
LEGEND: —□— = Control
—◆— = 1:100 Dilution of Anti-SC Antibody
—■— = 1:500 Dilution of Anti-SC Antibody
—○— = 1:1000 Dilution of Anti-SC Antibody

FIGURE 146

**WESTERN BLOT: ANTI-SECRETORY COMPONENT
BEST AVAILABLE COPY**

MW

109
kDa

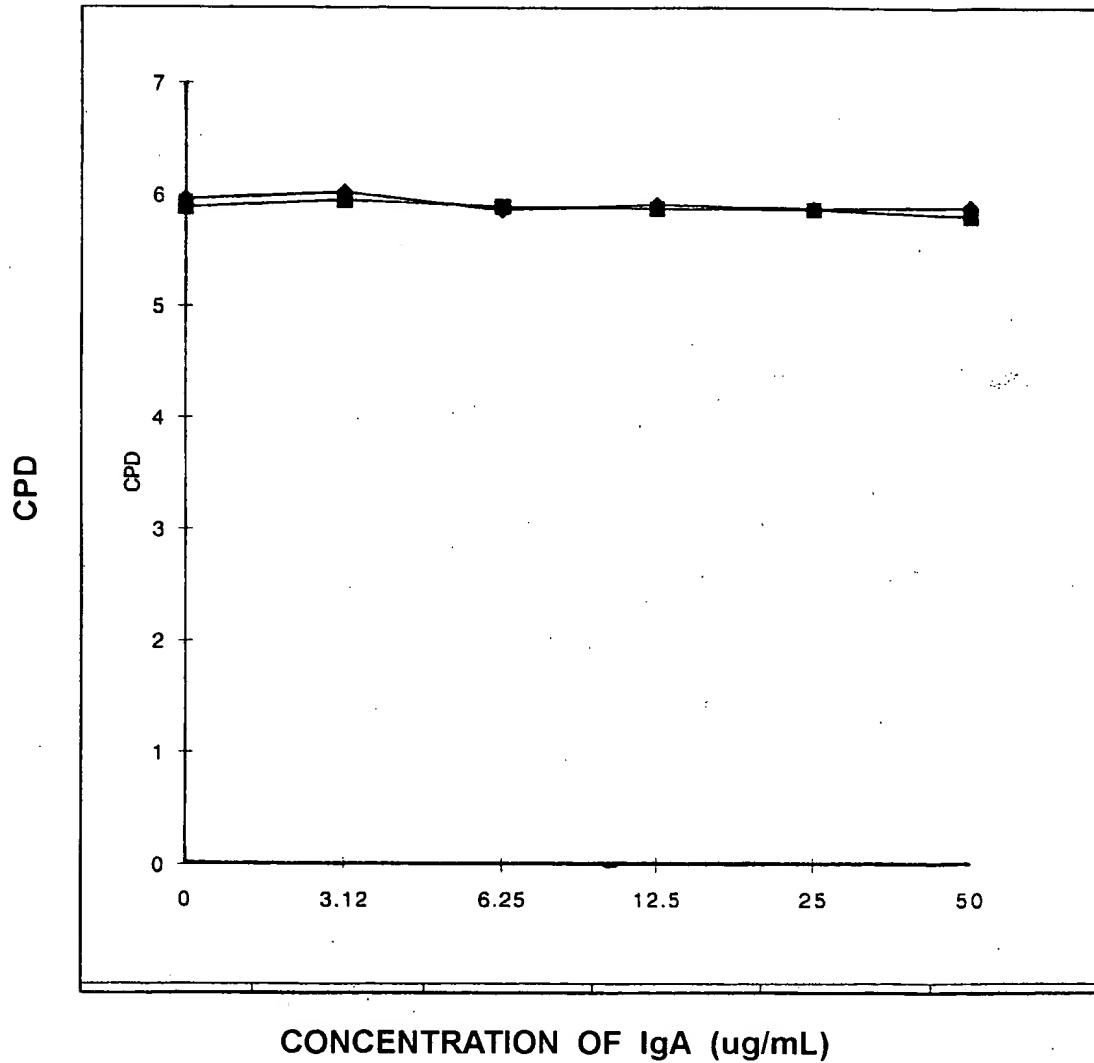


LEGEND:

1. MW
2. ALVA 41: 40 ug
3. ALVA 41: 20 ug
4. DU 145: 40 ug
5. DU 145: 20 ug
6. HUMAN FIBROBLAST: 40 ug
7. HUMAN FIBROBLAST: 20 ug
8. LNCaP: 40 ug
9. LNCaP: 20 ug
10. MDCK1: 20 ug
11. MDCK1: 10 ug
12. PC3: 40 ug

FIGURE 147

EFFECT OF HUMAN PLASMA IgA ON DU145
CELL GROWTH WITH AND WITHOUT DHT



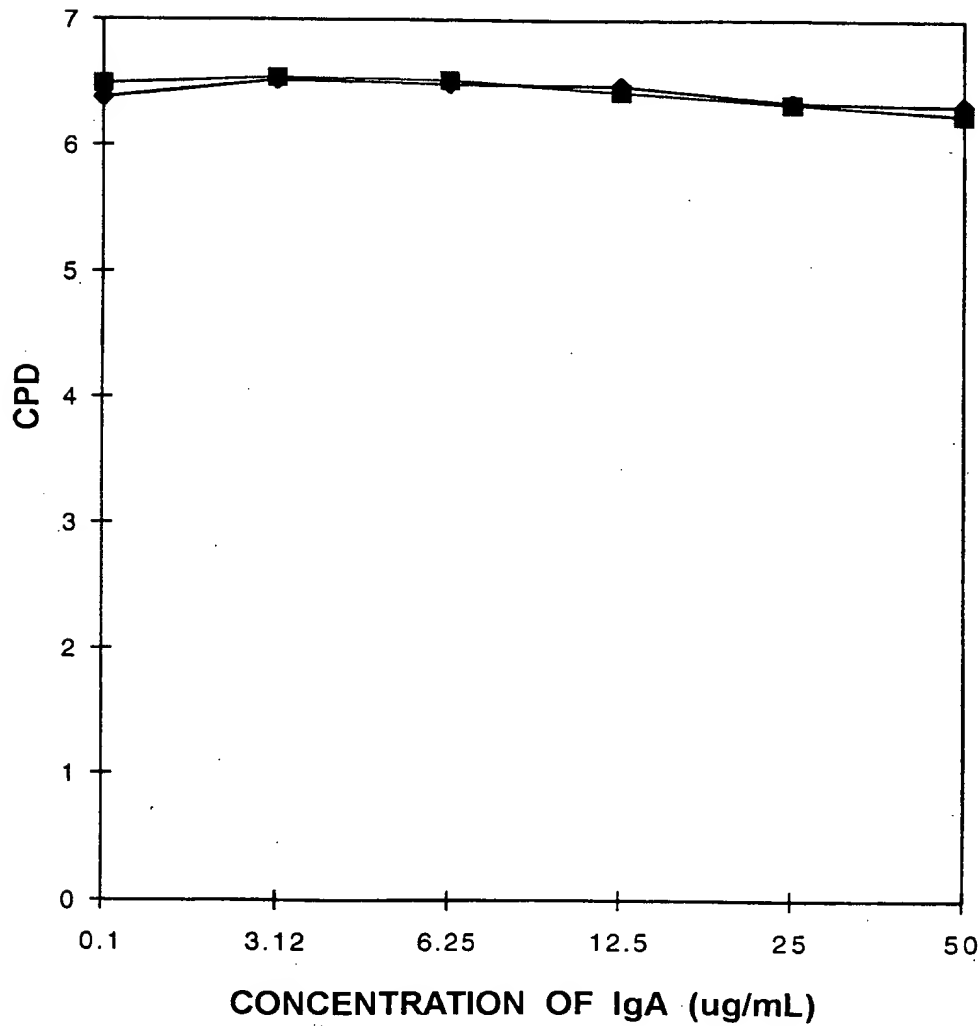
LEGEND:

—◆— = + DHT

—■— = - DHT

FIGURE 148

**EFFECT OF HUMAN PLASMA IgA ON PC3
CELL GROWTH WITH AND WITHOUT DHT**



LEGEND:

- ◆— = + DHT
—■— = - DHT